

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup>:</b> <b>A61K 9/10, 9/16</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 94/20072</b> <b>(43) International Publication Date:</b> 15 September 1994 (15.09.94)
<b>(21) International Application Number:</b> PCT/SE94/00185 <b>(22) International Filing Date:</b> 4 March 1994 (04.03.94) <b>(30) Priority Data:</b> 08/027,501      5 March 1993 (05.03.93)      US <b>(71) Applicant (for all designated States except US):</b> PHARMACIA AB [SE/SE]; S-171 97 Stockholm (SE). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> WESTESEN, Kirsten [DE/DE]; Am Klapperberge 4, D-3308 K�nigsutter (DE). SIEKMANN, Britta [DE/DE]; Isoldestrasse 30 B, D-3300 Braunschweig (DE). <b>(74) Agent:</b> HEDENSTR�M, John; Kabi Pharmacia AB, P.O. Box 941, S-251 09 Helsingborg (SE).	<b>(81) Designated States:</b> AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> SOLID LIPID PARTICLES, PARTICLES OF BIOACTIVE AGENTS AND METHODS FOR THE MANUFACTURE AND USE THEREOF  <b>(57) Abstract</b>  The present invention is in the area of administration forms and delivery systems for drugs, vaccines and other biologically active agents. More specifically the invention is related to the preparation of suspensions of colloidal solid lipid particles (SLPs) of predominantly anisometrical shape with the lipid matrix being in a stable polymorphic modification and of suspensions of micron and submicron particles of bioactive agent (PBAs); as well as to the use of such suspensions or the lyophilizates thereof as delivery systems primarily for the parenteral administration of preferably poorly water-soluble bioactive substances, particularly drugs, and to their use in cosmetic, food and agricultural products. The emulsification process includes melting of the agent (a lipid and for bioactive agent) and mixing with an aqueous phase (heated to melt temperature). The emulsification is completed by a high-pressure homogenization.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

## SOLID LIPID PARTICLES, PARTICLES OF BIOACTIVE AGENTS AND METHODS FOR THE MANUFACTURE AND USE THEREOF

5 This invention relates to suspensions of particles of biodegradable lipids solid at room temperature, preferably triglycerides, which can be used as carriers for poorly water soluble drugs or other bioactive agents, and to suspensions of particles constituted by biologically active agents such as drugs, insecticides, fungicides, pesticides, herbicides and fertilizers, as well as to the lyophilizates thereof. Both systems can be prepared by a  
10 melt emulsification process.

The properties of the solid lipid particles (SLPs) include biodegradability, avoidance of toxicologically active residues from the production process, enhanced physicochemical stability with regard to coalescence and drug leakage, modified surface characteristics, controlled release of in-  
15 corporated substances and modified biodistribution. The particles can be prepared by an emulsification process of molten material creating liquid droplets which form crystalline anisometrical particles on cooling. The anisometrical particles are of micron and submicron size, predominantly in the size range from 50 to 500 nm. The described suspensions have several  
20 advantages over other drug carrier systems deriving from the solid biodegradable matrix being predominantly present in a  $\beta$ -polymorphic modification (e.g.  $\beta'$ ,  $\beta_1$ ,  $\beta_2$ ), and not in an amorphous or  $\alpha$ -crystalline state.

The preparation of micron and submicron particles consisting of poorly water-soluble bioactive agents (PBAS) by emulsification of the  
25 molten substance presents a novel process to reduce the particle size and/or to modify the surface characteristics of powdered substances which can be accomplished by inexpensive techniques and by the use of physiologically acceptable additives only. The suspensions of the particles are an easy-to-handle product from the security point of view. The particles of  
30 bioactive agents provide for the modified biodistribution and bioavailability of the formulated drugs or other bioactive substances which implies a modification of the extent and rate of dissolution and absorption, the circulation time, the site of action and the way of disposition of the drug or

other bioactive substance. A reduction in particle size below the micrometer range provides for the direct intravenous administration of particles made from poorly water-soluble drugs without the need of a carrier vehicle.

## 5 FIELD OF THE INVENTION

The present invention is in the area of administration forms and delivery systems for drugs, vaccines and other biologically active agents such as insecticides, fungicides, pesticides, herbicides and fertilizers. More specifically, the invention is related to the preparation of suspensions of  
10 colloidal solid lipid particles (SLPs) with the lipid matrix being in a stable polymorphic modification and of suspensions of micron and submicron particles of bioactive agents (PBAs), as well as to the use of such suspensions or the lyophilizates thereof as delivery systems, primarily for the parenteral but also for the peroral, nasal, pulmonary, rectal, dermal and  
15 buccal administration of preferably poorly water-soluble bioactive substances, particularly drugs; and to their use in cosmetic, food and agricultural products. These suspension systems provide for the controlled release of incorporated or constituting substances as well as for the modified biodistribution and bioavailability of incorporated or constituting drugs,  
20 which implies a modification of the extent and rate of dissolution and absorption, the circulation time, the site of action and the way of disposition of the drug.

## BACKGROUND OF THE INVENTION

25 The parenteral, in particular the intravenous administration of water-insoluble or poorly water-soluble substances such as drugs or other biological materials often presents a problem to the formulator. Since the diameter of the smallest blood capillaries is only a few microns the intravenous application of larger particles would lead to capillary blockage. Solid  
30 drug substances are, however, commonly disintegrated by milling and grinding, thereby generating particles from a few millimeters down to the micrometer size range which are too large to be injected directly as an aqueous suspension. As a consequence, intravenous administration

systems containing suspended particles of water-insoluble drugs are not commercially available due to the risk of embolism. A further decrease in particle size is expensive, ineffective or even impossible by conventional techniques. Additionally, the reduction of solids to submicron-sized powders  
5 brings about heavy difficulties in handling these dry products such as an increased risk of dust explosions and cross-contamination problems in a factory environment. Moreover, such systems present a health risk for persons exposed to the possible inhalation and absorption of potent bio-active materials. Up to now the only possibilities to administer poorly  
10 water-soluble substances by the intravenous route are the use of co-solvents or the development of carrier systems which incorporate such substances in vehicles with hydrophilic surfaces.

Basic requirements of an ideal drug carrier system imply biodegradability, non-toxicity and non-immunogenicity. Moreover, the carrier should be  
15 suitable for the intended route of administration, e g with regard to particle size. Often a controlled release of the incorporated bioactive material is desired, for example when constant serum levels should be maintained over a long period of time or when the drug exhibits only a low therapeutic index.

Furthermore, carrier systems can be employed to prolong the half-life  
20 of certain substances which are unstable due to rapid enzymatic or hydrolytic degradation in biological milieu. On the other hand the incorporation of drug in the carrier material also presents an opportunity to protect the host from the drug in case of non-selective toxic substances such as antitumour agents.

25 In many cases drug carrier systems are developed with the object to deliver drugs to site-specific targets under circumvention of uptake by the reticuloendothelial system (RES). The rationale for such a drug targeting is an enhancement of the drug's therapeutic efficacy by an increase of the drug concentration at the target site with a simultaneous decrease at non-  
30 target sites, thereby rendering possible a reduction of the administered dose. Thus, the toxicity of drugs, e g anticancer agents, can be diminished, leading to a decrease of side effects.

The prerequisite of a successful site-specific delivery implies a certain selectivity of the carrier system for the target tissue as well as the accessibility of the desired target site. Targeting by the intravenous route of application is generally connected to an avoidance or at least a reduction of carrier uptake by the RES except for the cases where a direct targeting to cells of the RES is desired. Clearance of colloidal particles by the RES has been described to depend on particle size as well as on particle surface characteristics such as surface charge and surface hydrophobicity. In general, small particles are cleared less rapidly from the blood stream than large particles whereas charged particles are taken up more rapidly than hydrophilic non-charged particles. Due to these facts approaches to drug targeting are the modification of surface characteristics and the reduction of particle size.

Moreover, a small particle size is also required for the targeting of drugs to extravascular sites since extravasation is only feasible through a receptor-mediated uptake by phagocytosis/pinocytosis or where the endothelial wall is fenestrated. These fenestrations can be found for example in the sinusoids of liver, spleen and bone-marrow and show diameters of up to approximately 150 nm.

From the manufacturing point of view the ideal drug carrier system should be preparable without complications by easy-to-handle techniques in a reproducible manner and possibly at low production costs. The formulation should exhibit sufficient stability during preparation as well as on storage.

In recent years several colloidal systems have received special interest for their potential application as drug carriers, among them being liposomes, lipid emulsions, microspheres and nanoparticles. However, all of the systems mentioned possess a certain number of draw-backs which so far have prevented the break-through of any such system as a wide-spread, commercially exploited drug carrier.

Drug carrier systems in the micrometer size range are represented by microspheres consisting of a solid polymer matrix, and microcapsules in which a liquid or a solid phase is surrounded and encapsulated by a

polymer film. Nanoparticles consist, like microspheres, of a solid polymer matrix. Their mean particle size, however, lies in the nanometer range. Both micro- and nanoparticles are generally prepared either by emulsion polymerization or by solvent evaporation techniques. Due to these production methods micro- and nanoparticles bear the risk of residual contaminations from the production process like organic solvents such as chlorinated hydrocarbons, as well as toxic monomers, surfactants and cross-linking agents, which may lead to toxicological problems. Moreover, some polymeric materials such as polylactic acid and polylactic-glycolic acid degrade very slowly in vivo so that multiple administration could lead to polymer accumulation associated with adverse side effects. Other polymers such as polyalkylcyanoacrylates release toxic formaldehyde on degradation in the body.

Drug carrier systems for parenteral administration based on lipids are liposomes and submicron lipid emulsions. Although such systems consist of physiological components only, thus reducing toxicological problems there is a number of disadvantages associated with these lipid carriers.

Liposomes are spherical colloidal structures in which an internal aqueous phase is surrounded by one or more phospholipid bilayers. The potential use of liposomes as drug delivery systems has been disclosed inter alia in the U.S. Pat Nos 3,993,754 (issued Nov 23, 1976 to Rahmann and Cerny), 4,235,871 (issued Nov 25, 1980 to Papahadjopoulos and Szoka) and 4,356,167 (issued Oct 26, 1982 to L. Kelly). The major drawbacks of conventional liposomes are their instability on storage, the low reproducibility of manufacture, the low entrapment efficiency and the leakage of drugs.

According to the IUPAC definition, in an emulsion liquids or liquid crystals are dispersed in a liquid. Lipid emulsions for parenteral administration consist inter alia of liquid oil droplets, predominantly in the submicron size range, dispersed in an aqueous phase and stabilized by an interfacial film of emulsifiers. Typical formulations are disclosed in the Jap. Pat. No 55,476/79 issued May 7, 1979 to Okamoto, Tsuda and Yokoyama. The preparation of a drug containing lipid emulsion is described in WO



91/02517 issued March 7, 1991 to Davis and Washington. The susceptibility of these lipid emulsions towards the incorporation of drugs is relatively high due to the mobility of drug molecules within the internal oil phase since diffusing molecules can easily protrude into the emulsifier film causing instabilities which lead to coalescence. Furthermore, release of incorporated drugs from lipid emulsions is relatively fast so that the possibilities for a sustained drug release are limited.

Fountain et al (U.S. Pat. No 4,610,868 issued Sept. 9, 1986) developed lipid matrix carriers which are described as globular structures of a hydrophobic compound and an amphiphatic compound with diameters from about 500 nm to about 100.000 nm. The hydrophobic compound can be liquid or solid. The preparation techniques, however, employ organic solvents and are thus associated with the problem of complete solvent removal.

So-called lipospheres disclosed by Domb et al (U.S. Pat. Appl. No 435,546 lodged Nov 13, 1989; Int. Appl. No PCT/US90/06519 filed Nov 8, 1990) are described as suspensions of solid, water-insoluble microspheres made of a solid hydrophobic core surrounded by a phospholipid layer. Lipospheres are claimed to provide for the sustained release of entrapped substances controlled by the phospholipid layer. They can be prepared by a melt or by a solvent technique, the latter creating toxicological problems if the solvent is not completely removed.

A slow release composition of fat or wax and a biologically active protein, peptide or polypeptide suitable for parenteral administration to animals is disclosed in U.S. Pat. Appl. No 895,608 lodged Aug 11, 1986 to Staber, Fishbein and Cady (EP-A-0 257 368). The systems are prepared by spray drying and consist of spherical particles in the micrometer size range up to 1.000 microns so that intravenous administration is not possible.

Problems with the formulation of water-insoluble or poorly water-soluble substances are not restricted to the parenteral route of administration. Thus, the peroral bioavailability of drugs is related to their solubility in the gastrointestinal tract (GIT), and it is generally found that poorly water-soluble drugs exhibit a low bioavailability. Moreover, the dissolution of

drugs in the GIT is influenced by their wettability. Substances with apolar surfaces are scarcely wetted in media so that their dissolution rate is very slow.

In an attempt to improve the intestinal absorption of lipophilic drugs, Eldem et al (Pharm. Res. 8, 1991, 47-54) prepared lipid micropellets by spray-drying and spray-congealing processes. The micropellets are described as spherical particles with smooth surfaces. The lipids are, however, present in unstable polymorphic forms, and polymorphic phase transitions occur during storage so that the product properties are constantly changing (T. Eldem et al, Pharm. Res. 8, 1991, 178 -184). Thus, constant product qualities cannot be assured.

Lipid nanopellets for peroral administration of poorly bioavailable drugs are disclosed in EP 0 167 825 of Aug 8, 1990 to P. Speiser. The nanopellets represent drug-loaded fat particles solid at room temperature and small enough to be persorbed. Persorption is the transport of intact particles through the intestinal mucosa into the lymph and blood compartment. The lipid nanopellets are prepared by emulsifying molten lipids in an aqueous phase by high-speed stirring. After cooling to room temperature the pellets are dispersed by sonication.

## OBJECT OF THE INVENTION

Considering the limitations of conventional drug carriers such as liposomes, lipid emulsions, nanoparticles and microspheres as outlined above there is an obvious demand for a carrier system for the controlled delivery of poorly water-soluble bioactive substances to circumvent the drawbacks of traditional systems particularly with regard to preparation, stability, toxicity and modification of biodistribution.

The present invention introduces a new type of carrier system characterized as non-spherically shaped particles composed of crystalline lipids, preferably triglycerides, and physiologically acceptable additives as well as a process for the manufacturing thereof. These carriers provide for the controlled delivery of poorly water-soluble substances such as drugs or other biological materials primarily by the parenteral but also by the peroral,

nasal, pulmonary, rectal, dermal and buccal route of administration, and will hereinafter be referred to as solid lipid particles (SLPs).

SLPs are characterized as lipidic particles of a solid physical state in the micro- and predominantly in the nanometer size range. The shape of the particles is mainly anisometric which is a consequence of the matrix forming lipids present in a  $\beta$ -polymorphic modification (e.g.  $\beta'$ ,  $\beta_1$ ,  $\beta_2$ ), and not in an amorphous or  $\alpha$ -crystalline state. The properties of the SLPs include: (1) biodegradability and non-toxicity; (2) the ability to incorporate poorly water-soluble substances; (3) improved chemical and physical stability; (4) the possibility to prepare a dry storage formulation; (5) control of release characteristics of incorporated substances; and (6) modified surface characteristics. As a result of these properties SLPs overcome many of the problems encountered with conventional drug carrier systems.

The present invention is supposed to bring about the following advantages as derived from the characteristics of the SLPs described above:

- (1) SLPs can be prepared of biodegradable, pharmacologically acceptable compounds only and are, therefore, non-toxic. Additionally, the preparation of SLPs avoids the employment of organic solvents or any other potentially toxic additives, thus evading the contamination of the product with residual impurities.
- (2) SLPs possess an enhanced chemical stability as compared with conventional lipid emulsions based on liquid triglyceride oils owing to the lower degree of unsaturated fatty acids of solid triglycerides. Moreover, SLPs exhibit a better physical stability due to the solid nature of the lipid matrix which is expectedly more resistant to coalescence than fluid emulsion droplets. Furthermore, the lipid matrix is present in a stable  $\beta$ -polymorphic modification (e.g.  $\beta'$ ,  $\beta_1$ ,  $\beta_2$ ). Thus, the product properties will not change significantly during long-term storage due to polymorphic transformations.

- 5 (3) Suspensions of SLPs can be lyophilized by freeze-drying to provide a water-free storage system that exhibits a good long-term stability. The lyophilized powder can be redispersed in water, buffer or solutions of amino acids, carbohydrates and other infusion solutions directly before use or can be processed into other pharmaceutical formulations.
- 10 (4) Due to their lipophilic nature SLPs are suited for the solubilization of lipophilic and poorly water-soluble substances by entrapment into the lipid matrix. Compared to lipid emulsions SLPs are supposed to be less sensitive to the incorporation of drugs or other bioactive materials due to their solid nature. Drugs or other bioactive materials diffusing into the emulsifier film or recrystallizing close to the surface perturb the stabilizing film of emulsion droplets, increasing the risk of film rupture followed by coalescence. In contrast, film elasticity and film viscosity are of minor importance in the case of solid suspension particles since they cannot coalesce because of the rigid nature of the lipid.
- 15 (5) Drug release from the lipid carrier can be controlled for example by the composition of the lipid matrix by the choice of stabilizing agents as well as by the size of SLPs. Drug leakage is hindered by the solid state of the carrier due to the restricted drug diffusion.
- 20 (6) Drugs or bioactive substances exhibiting a short half-life due to enzymatic or hydrolytic degradation can be protected from rapid decomposition by incorporation within the lipid carrier since the hydrophobic matrix prevents the access of water to the incorporated drug on storage as well as in body fluids.
- 25 (7) The incorporation into SLPs of drugs or other bioactive substances with a low bioavailability due to poor solubility in the gastrointestinal tract (GIT) can enhance the bioavailability of such substances because these are solubilized in the
- 30

biodegradable lipid matrix and are thus present in the dissolved state.

(8) Due to the anisometrical shape of SLPs the specific surface area is larger than that of spherical particles of the same volume. Substances with a low peroral bioavailability can be absorbed faster and to a higher degree in the GIT when they are incorporated in anisometrical SLPs than in spherical lipid particles of the same volume, due to the larger surface area of SLPs since the potential site of action for lipolytic enzymes is larger.

(9) The surface characteristics of SLPs can be modified by variation of the lipid composition, use of different stabilizers, exchange of surfactants and/or adsorption of polymeric compounds. The modification of surface characteristics brings about the possibility to modify the in vivo distribution of the carrier and the incorporated substance. In case of intravenous administration this implies a modified uptake by the RES with the potential for drug targeting.

(10) Due to the submicron particle size of SLPs there is no risk of embolism by parenteral administration. Since SLPs can be prepared down to a particle size of about 50 nm, they possess the opportunity of extravasation through fenestrations of the endothelial wall. Thereby, drugs can be targeted to extravascular sites such as the bone marrow, for example.

Furthermore, the present invention introduces a new type of delivery system for the parenteral, peroral, nasal, pulmonary, rectal, dermal and buccal administration of drugs or other bioactive substances as well as the process for the manufacturing thereof. These formulations are suspensions of particles formed by bioactive substances with modified surface characteristics and/or a reduced particle size as compared to the powdered substance, and will hereinafter be referred to as particles of bioactive agents (PBAs). The preparation of PBAs can avoid the employment of any

toxicologically active additives such as organic solvents or toxic monomers, and can be accomplished by easy-to-handle techniques.

PBAs can be used in the following fields of application:

- 5 a) as a parenteral delivery system with modified biodistribution for sparingly water-soluble bioactive substances without the need of a carrier vehicle;
- b) as a delivery system according to a) for peroral, nasal, pulmonary, rectal, dermal and buccal administration;
- 10 c) as a formulation for the peroral administration of drugs with a poor bioavailability due to a low dissolution rate in the gastrointestinal tract;
- d) as a delivery system for use in agricultural applications;
- e) the lyophilizate of formulations a) to d) as a reconstitutable powder with an enhanced stability on storage.

15 Owing to the special characteristics of the present invention PBAs are supposed to bring about the following advantages over conventional pharmaceutical delivery systems:

- 20 1) The formulation of poorly water-soluble drugs or other bioactive substances as micron and submicron particles avoids the need of a carrier system for their parenteral application, thereby circumventing the disadvantages of conventional drug carriers like liposomes, lipid emulsions, nanoparticles and microspheres.
- 25 2) PBAs can be prepared by easy-to-handle techniques in a reproducible way. There are no problems to foresee for the scaling up of the manufacturing process.
- 3) Since the particles consist of the pure bioactive compound with only small amounts of stabilizers the drug-load capacity of the drug particles is high.
- 30 4) The release of drugs or other bioactive compounds from the formulation can be controlled by the choice of amphiphatic compounds employed to stabilize the particles.
- 5) The preparation of PBAs can avoid the use of toxicologically active additives.

- 6) A water-free storage system with enhanced stability can be produced, for example by freeze-drying of the PBA dispersions.
- 7) The surface characteristics of PBAs can be modified by the choice of amphiphatic compounds used as stabilizers as well as by the attachment of so-called homing devices for the targeting of drugs, for example monoclonal antibodies or carbohydrate moieties. The surface modifications give rise to a modified bioavailability and biodistribution with regard to the extent and rate of absorption, the circulation time, the site of action and the way of disposition of the bioactive substance. The modification of surface characteristics also provides the opportunity to avoid or at least to reduce the uptake of intravenously administered particles by cells of the RES.
- 8) Since the particles can be prepared with a size below 100 nm to 200 nm they possess the opportunity for extravasation by fenestrations of the endothelial wall. Thereby, drugs can be targeted to extravascular sites such as the bone-marrow, for example.
- 9) A reduction in particle size to the nanometer size range generally not achievable by milling or grinding leads to an enormous increase of the specific surface area of the particles. Since the peroral bioavailability of drugs or other bioactive substances is related to the specific surface area via the dissolution rate of the substance in the gastrointestinal tract the submicron sized particles give rise to an enhanced bioavailability of drugs poorly soluble in the GIT.
- 10) Hydrophobic substances can be formulated as PBAs with hydrophilic surfaces. Hydrophilic surfaces provide for a good wettability of the particles, for example in the GIT, facilitating dissolution of the compound. Thus, the bioavailability can be increased.
- 11) The process of manufacturing of PBAs involves inexpensive easy-to-handle techniques only and provides a product which is safe to handle. Since the particles are present in a liquid dispersion there is no risk of dust explosions, cross-contamination or inhalation of bioactive substances as often encountered with the production of extremely fine powders.

## DESCRIPTION OF THE INVENTION

The present invention relates to suspensions of micron and sub-micron particles of biodegradable lipids solid at room temperature (solid lipid particles, SLPs), to suspensions of particles of meltable bioactive substances (PBAs), to lyophilizates thereof and to methods for the manufacturing thereof.

Solid lipid particles (SLPs) are of predominantly anisometrical shape which is a consequence of the lipid matrix being present in a  $\beta$ -polymorphic modification (e.g.  $\beta'$ ,  $\beta_1$ ,  $\beta_2$ ) or in a polymorphic state analogous to that of  $\beta$ -crystals of triglycerides and not in an amorphous or  $\alpha$ -crystalline-like state. SLPs can be used as carrier systems primarily for the parenteral but also for the peroral, nasal, pulmonary, rectal, dermal and buccal administration of poorly water-soluble substances such as drugs or other biologically active materials. The application of SLPs is, however, not restricted to the administration of pharmaceuticals to humans or animals. SLPs can also be used in cosmetic, food and agricultural products. SLPs are novel lipid structures with properties that overcome many of the problems associated with previously described carrier systems.

The matrix of SLPs is constituted by biocompatible hydrophobic materials which are solid at room temperature and have melting points ranging from approximately 30 to 120°C. The preferred matrix constituents are solid lipids (fats) such as mono-, di- and triglycerides of long-chain fatty acids; hydrogenated vegetable oils; fatty acids and their esters; fatty alcohols and their esters and ethers; natural or synthetic waxes such as beeswax and carnauba wax; wax alcohols and their esters, sterols such as cholesterol and its esters, hard paraffins, as well as mixtures thereof. The carrier material must be compatible with the agent to be incorporated.

Lipids are known to exhibit a pronounced polymorphism. This can be defined as the ability to reveal different unit cell structures in crystal, originating from a variety of molecular conformations and molecular packings. Depending on the conditions, glycerides, for example, may crystallize in three different polymorphic forms termed alpha ( $\alpha$ ), beta prime ( $\beta'$ ) and beta ( $\beta$ ) according to the classification of Larsson (K. Larsson, 1966, Acta Chem.



Scand. 20, 2255-2260). These polymorphic modifications characterized by a particular carbon chain packing may differ significantly in their properties such as solubility, melting point and thermal stability. Transformations take place from  $\alpha$  to  $\beta'$  to  $\beta$ , the transition being monotropic. The  $\beta$ -form is the thermodynamically most stable polymorph, whereas  $\alpha$  is the least stable and will transform more or less rapidly into the more stable polymorphs  $\beta'$  and  $\beta$ , depending on the thermal conditions. This transformation is accompanied by a change of physicochemical properties.

In the described suspensions of SLPs the lipid matrix is predominantly present in a stable polymorphic modification. Although on cooling, the dispersed melt metastable polymorphs such as the  $\alpha$ -form may occur intermediately a stable polymorph is formed within several hours or days after preparation of the dispersions.

The suspensions of SLPs can be stabilized by amphiphatic compounds such as ionic and non-ionic surfactants. Suitable stabilizers include but are not limited to the following examples: naturally occurring as well as synthetic phospholipids, their hydrogenated derivatives and mixtures thereof, sphingolipids and glycosphingolipids; physiological bile salts such as sodium cholate, sodium dehydrocholate, sodium deoxycholate, sodium glycocholate and sodium taurocholate; saturated and unsaturated fatty acids or fatty alcohols; ethoxylated fatty acids or fatty alcohols and their esters and ethers; alkylaryl-polyether alcohols such as tyloxapol; esters and ethers of sugars or sugar alcohols with fatty acids or fatty alcohols; acetylated or ethoxylated mono- and diglycerides; synthetic biodegradable polymers like block co-polymers of polyoxyethylene and polyoxypropyleneoxide; ethoxylated sorbitanesters or sorbitanethers; amino acids, polypeptides and proteins such as gelatine and albumin; or a combination of two or more of the above mentioned.

The aqueous phase in which the SLPs are dispersed can contain water-soluble or dispersable stabilizers; isotonicity agents such as glycerol or xylitol; cryoprotectants such as sucrose, glucose, trehalose etc; electrolytes; buffers; antiflocculants such as sodium citrate, sodium pyrophosphate or sodium dodecylsulfate; preservatives.

Depending on the characteristics of the employed stabilizers the coexistence of other colloidal structures such as micelles and vesicles in suspensions of SLPs cannot be ruled out.

Substances particularly suitable for the entrapment into SLPs are  
5 drugs or other bioactive compounds which are poorly water-soluble, show a low bioavailability, are badly absorbed from the intestinum, and/or will be rapidly degraded in biological environment by chemical or enzymatical processes, as well as low-specific active substances which are highly toxic at non-target sites. In case it is desired to incorporate a relatively water-  
10 soluble compound into SLPs it is necessary to decrease the water-solubility of this compound, which can be achieved for example by using a water-insoluble derivative of the compound such as an acid or base, a complex, or a lipophilic precursor.

Drugs or bioactive agents particularly suited for incorporation into  
15 SLPs are antibiotics such as fosfomycin, fosmidomycin and rifapentin; antihypertensives such as minoxidil, dihydroergotoxine and endralazine; antihypotensives such as dihydroergotamine; systemic antimycotics such as ketoconazole and griseofulvin; antiphlogistics such as indomethacin, diclofenac, ibuprofen, ketoprofen and piroprofen; antiviral agents such as  
20 aciclovir, vidarabin and immunoglobulines; ACE inhibitors such as captopril and enalapril; betablockers such as propranolol, atenolol, metoprolol, pindolol, oxprenolol and labetalol; bronchodilators such as ipratropium-bromide and sobrerol; calcium antagonists such as diltiazem, flunarizin, verapamil, nifedipin, nimodipin and nitrendipin; cardiac glycosides such as  
25 digitoxin, digoxin, methyldigoxin and acetyldigoxin; cephalosporins such as ceftizoxim, cefalexin, cefalotin and cefotaxim; cytostatics such as chlor-methin, cyclophosphamid, chlorambucil, cytarabin, vincristin, mitomycin C, doxorubicin, bleomycin, cisplatin, taxol, penclomedine and estramustin; hypnotics such as flurazepam, nitrazepam and lorazepam; psychotropic  
30 drugs such as oxazepam, diazepam and bromazepam; steroid hormones such as cortisone, hydrocortisone, prednisone, prednisolone, dexamethasone, progesterone, pregnanolone, testosterone and testosteroneundecanoat; vasodilators such as molsidomin, hydralazin and dihydralazin;

cerebral vasodilators such as dihydroergotoxin, ciclonicat and vincamin;  
lipophilic vitamins such as vitamins A, D, E, K and their derivates.

The bioactive substances can be located in the core of SLPs where  
they are dissolved, solubilized or dispersed in the matrix, and/or in the  
5 stabilizer layer(s) surrounding the particle matrix; and/or can be adsorbed to  
the surface of SLPs. The bioactive substances can be dissolved or  
crystalline or amorphous or a mixture of these crystallographic states.

SLPs can be prepared by an emulsification process which exhibits  
certain similarities to the preparation of lipid(oil)-in-water emulsions but is  
10 mainly characterized by its basic differences as will be outlined below. The  
process is described as follows:

- (1) The solid lipid or the mixture of lipids is melted.
- (2) The stabilizers are added either to the lipid and to the dispersion  
medium or to the dispersion medium only, depending on their  
15 physicochemical characteristics. The choice of stabilizers and the  
admixture regime are not comparable with those applied for lipid  
(oil)-in-water emulsions, which is evident from the below examples.  
Stabilizers may also be added or exchanged after homogenization,  
for example by adsorption of polymers or by dialysis of water-soluble  
20 surfactants.
- (3) Drugs or other bioactive substances to be incorporated into the SLPs  
may be melted together with the lipids if the physicochemical  
characteristics of the substance permit, or may be dissolved, solu-  
bilized or dispersed in the lipid melt before homogenization.
- 25 (4) The dispersion medium is heated to the temperature of the melt  
before mixing and may contain for example stabilizers, isotonicity  
agents, buffering substances, cryoprotectants and/or preservatives.
- (5) The melted lipid compounds are emulsified in the dispersion medium,  
preferably by high pressure homogenization, but emulsification is  
30 also possible by sonication, high speed stirring, vortexing and vigor-  
ous hand shaking. The way of homogenization determines the  
particle size of the SLPs.

The basic differences to the preparation of lipid-in-water emulsions beside the choice and admixture regime of the stabilizers are related to the following steps:

- 5 (6) After homogenization the dispersion can be sterilized by standard techniques such as autoclaving or filtration through a 0.2  $\mu\text{m}$  sterile filter provided the particles are small enough not to be retained by the filter. These steps have to be performed before the system is cooled below the recrystallization temperature. Moreover, contaminations which could lead to heterogenous nucleation should be avoided.
- 10 ed. It is therefore advisable to remove particulate contaminations from the dispersions by filtration prior to cooling below the recrystallization temperature. The pore size of the filter should be chosen sufficiently large so as not to retain the lipid particles.
- 15 (7) The dispersions are allowed to stand to cool off at room temperature forming SLPs by recrystallization of the dispersed lipids. During cooling the dispersion may be agitated by a magnetic stirrer for example.
- 20 (8) In a subsequent step the dispersion medium is reduced in volume for example by evaporation or it can be removed by standard techniques such as filtration, ultrafiltration or freeze-drying, thus yielding a water-free storage system which can be reconstituted prior to use. The lyophilized powder can also be processed into other pharmaceutical, cosmetic, food or agricultural formulations such as powders, tablets, capsules etc.
- 25 SLPs are typically solid particles of anisometrical shape as demonstrated by Fig. 1 which shows a transmission electron micrograph of a freeze-fractured specimen of the SLPs of Example 1. The anisometrical particle shape results from crystallization of the lipid matrix in the  $\beta$ -polymorphic form. Solidification of the amorphous fat or crystallization of the
- 30 unstable  $\alpha$ -polymorph generally reveals spherical particles. The presence of the stable  $\beta$ -form could be detected by differential scanning calorimetry (Fig. 2) and synchrotron radiation wide-angle X-ray diffraction (Fig. 3).

The particle size of SLPs depends on the type and amount of emulsifier and on the emulsification technique and conditions (see below). The resolidification of the molten lipids prior to homogenization should be avoided because size reduction by homogenization is substantially impeded if the particles are solid during this step. To ensure that the molten lipids do not solidify prior to homogenization, i.e. before smaller particles can be formed, the dispersion medium is heated to approximately the same temperature as the melt before the two phases are mixed so that the melt will not be cooled down by the addition of the dispersion medium.

SLPs in the nanometer size range are obtained by high-pressure homogenization. The particles show a relatively narrow particle size distribution with mean particle sizes by number of approximately 50 - 300 nm as determined by photon correlation spectroscopy (PCS). The dispersions of SLPs are stable on storage for more than 18 months. Thus, the long-term stability is similar to that of submicron o/w emulsions used for parenteral nutrition. Long-term stability data of other solid lipid-based carrier dispersions described in the patent literature such as lipospheres (A. Domb et al, Int. Appl. No PCT/US90/06519 filed Nov 8, 1990) and lipid nanopellets (P. Speiser, Eur. Pat. No 0167825 issued Aug 8, 1990) could not be found. Domb describes phospholipid stabilized tristearate lipospheres with a seven day stability as "exceptionally stable".

It turned out that the stabilization of SLP suspensions requires the presence of a highly mobile stabilizing agent in the dispersion medium in such a way that the amount of highly mobile stabilizers in the dispersion medium is, after emulsification, sufficient to stabilize newly created surfaces during recrystallization (see below). Bile salts, especially in combination with nonelectrolytic compounds such as glycerol in concentrations used to achieve blood isotony, have proved to be very efficient in this respect. SLPs stabilized by phospholipids alone, or in combination with nonelectrolytic compounds such as glycerol in concentrations used to achieve blood isotony, tend to form semi-solid ointment-like gels as shown on the transmission electron micrograph of Fig. 4, whereas the addition of sodium

glycocholate to the aqueous phase prevents this gel formation (B. Siekmann and K. Westesen, 1992, Pharm. Pharmacol. Lett. 1, 123-126).

5 A molar phospholipid to bile salt ratio between 2:1 and 4:1 turned out to be most effective regarding the initial stabilization during homogenization as well as the long-term storage stability of SLP dispersions. These phospholipid/bile salt ratios are above the ratio of formation of mixed micelles and coincide with a swollen lamellar phase of mixed lecithin/bile salt layers in the ternary phase diagram of the system lecithin/bile salt/water. Data  
10 therefore suggest that stabilization is most effective if the bile salt is not bound to mixed micelles and that during stabilization of SLPs the bile salt molecules are inserted in the phospholipid layer on the surface of the particles.

SLPs can also be sterically stabilized by nonionic surfactants. Steric stabilization of SLPs requires, however, a relatively high amount of surfactants with lipid/surfactant ratios up to 1:1. It can be observed in general that  
15 the stability of SLPs decreases with increasing lipid/surfactant ratio.

The amount of emulsifier required for surface stabilization of the dispersed particles is higher than in conventional lipid emulsions, for example such as used in parenteral nutrition. This effect can be attributed  
20 to the crystallization of the molten lipids after homogenization. Since the lipids typically do not recrystallize or exist (on storage) in the form of ideal spheres but as anisometric particles there is a large increase in surface area as compared to the droplets of the emulsified molten lipids or of conventional lipid emulsions, respectively. The additional surfaces newly  
25 generated during recrystallization or polymorphic transitions of the dispersed lipids need to be stabilized immediately on formation to avoid particle aggregation. Therefore, the preparation of stable SLP dispersions requires the presence of a reservoir of stabilizing agents after emulsification.

The choice of stabilizers cannot be deduced from compositions and  
30 stabilization mechanisms for oil-in-water emulsions but is dependent on the existence of highly mobile stabilizers due to the formation mechanism of the anisometrical particles. In colloid and surface science "highly mobile" generally refers to free diffusion in the dispersion medium at a high diffusion

velocity. With regard to the stabilization of colloidal solid lipid particles the diffusion velocity should be sufficiently high to reach freshly created particle surfaces (especially during re-crystallization of the lipid) before particle aggregation can take place, in order to exert a stabilizing action at the lipid/water interface to prevent particle aggregation. Sufficiently high diffusional velocities are typically observed with substances which do not form a separate phase in the dispersion medium according to the phase rule set up by Gibbs. Highly mobile stabilizers can be of ionic or nonionic nature. Typically, these stabilizers dissolve molecularly in the dispersion medium and/or form micelles. Micelles are known to be highly dynamic structures characterized by a fast exchange of molecules between micellar aggregates and the dispersion medium. The monomers in the dispersion medium are immediately available for surface stabilization. In contrast, stabilizing agents that tend to form a separate phase in the dispersion medium are not sufficiently mobile to stabilize freshly created surfaces before particle aggregation can take place. These stabilizers are therefore not suitable as sole stabilizers of SLP dispersions. Phospholipids are an example of stabilizing agents that form a separate phase in the dispersion medium. It is well known that phospholipids form closed lamellar structures, so called vesicles, in aqueous media, and that the exchange rate of phospholipid molecules between vesicles and the aqueous phase is extremely small compared to that of micelles. Phospholipid molecules are therefore bound in vesicular structures and are not immediately available to stabilize newly created surfaces during recrystallization of the lipid particles. Consequently, phospholipids alone, although suitable as effective stabilizers of lipid emulsions, cannot efficiently stabilize SLP suspensions as is evident from Examples 5 and 13. In fact, preparing SLPs with a standard composition of lipid emulsions, for example 10% fat and 1.2% phospholipids, results in unstable SLP dispersions. Even higher concentrations of phospholipids such as 20% or 60% lecithin related to the fat phase are not sufficient to stabilize SLP dispersions as demonstrated in Example 5.

Although stated in the patent literature (e.g. Domb et al in U.S. Pat No 435,546 issued Nov 13, 1989 and Int. Appl. No PCT/US90/06519 filed

Nov 8, 1990), fine suspensions of solid lipids are not equivalent to sub-micron lipid emulsions in that respect that the inner phase is only replaced by solid fats instead of liquid ones. The physicochemical properties of lipid suspensions such as SLPs differ substantially from that of lipid emulsions.

5 As a consequence of these differences lipid suspensions cannot be prepared and treated analogously to lipid emulsions. One basic difference of SLPs is their much larger particle surface area as outlined above so that SLPs require a higher amount of surfactants which additionally need to be highly mobile to immediately stabilize the new surfaces created when the  
10 molten lipid recrystallizes or transforms into the stable  $\beta$ -polymorph. The second basic difference is that once SLPs are formed by recrystallization of the molten lipid any renewed melting of the small particles may result in an instability of the dispersions if there is no excess of mobile stabilizer in the aqueous phase which is not adsorbed to particle surfaces where it is  
15 immobilized. A further requirement for physicochemical stability of SLPs in contrast to oil-in-water emulsions is the absence of particulate impurities which could promote heterogenous nucleation. It is therefore advisable to remove particulate contaminations from the dispersions by filtration prior to cooling below the recrystallization temperature. Moreover, non-electrolytic  
20 compounds used to achieve blood isotony such as glycerol turned out to promote the stability of SLP dispersions.

The present invention also relates to suspensions of particles of bioactive agents (PBAs). Sparingly water-soluble substances such as drugs, insecticides, fungicides, pesticides, herbicides, fertilizers, nutrients, cos-  
25 metics etc which are meltable in the temperature range from approximately 30 to 120°C can be formulated as PBAs by a procedure similar to the preparation of SLPs as described above. The matrix of PBAs is constituted by the bioactive agent itself.

PBAs present a novel type of delivery system and can be charac-  
30 terized as predominantly submicron and/or micron particles of bioactive agents suspended in an aqueous media stabilized by amphiphatic compounds. PBAs possess modified surface characteristics which can be controlled by the choice of amphiphiles and/or a reduced particle size of the



matrix constituting compound as compared to the powdered substance. These characteristics give rise to a modified biodistribution and bioavailability of the formulated drugs or other bioactive substances which implies a modification of the extent and rate of dissolution and absorption, the circulation time, the site of action and the way of disposition of the drug or other bioactive substance. The physicochemical properties of PBAs depend strongly on the characteristics of the bioactive agent of which they are formulated, on the type and amount of stabilizing agents as well as on the way of emulsification. Suspensions and lyophilizates of PBAs can be used for the peroral, nasal, pulmonary, rectal, dermal, buccal and, depending on the particle size, also for the parenteral administration of poorly water-soluble drugs or other biologically active compounds. Moreover, PBAs can also be employed in cosmetic, food and agricultural products, in particular for the formulation of poorly water-soluble herbicides and pesticides.

The matrix of PBAs is constituted by practically insoluble or sparingly water-soluble agents with melting points preferably below 100°C or the melting points of which can be decreased to below 100°C by the addition of certain adjuvants. Substances particularly suitable for the formulation as PBAs are drugs or other bioactive materials which are poorly water-soluble, show a low bioavailability and/or are badly absorbed from the intestinum. Examples of such substances comprise but are not limited to:

Anesthetics and narcotics such as butanilcaine, fomicaine, isobutambene, lidocaine, risocaine, prilocaine, pseudococaine, tetracaine, trimecaine, tropacocaine and etomidate; anticholinergics such as metixen and profenamine; antidepressives, psychostimulants and neuroleptics such as alimenazine, binedaline, perazine, chlorpromazine, fenpentadiol, fenanisol, fluanisol, mebenazine, methylphenidate, thioridazine, toloxaton and trimipramine; antiepileptics such as dimethadion and nicethamide; antimycotics such as butoconazole, chlorphenesin, etisazole, exalamid, pecilocine and miconazole; antiphlogistics such as butibufen and ibuprofen; bronchodilators such as bamifylline; cardiovascular drugs such as alprenolol, butobendine, cloridazole, hexobendine, nicofibrate, penbutolol, pirmenol, prenylamine, procaine amide, propatylnitrate, suloctidil, toliprolol,

xibendol and viquidile; cytostatics such as asperline, chlorambucile, chlor-naphhazine, mitotane, estramustine, taxol, penclomedine and trofosfamide; hyperemic drugs such as capsaicine and methylnicotinate; lipid reducers such as nicoclonate, oxprenolol, pirifibrate, simfibrate and thiadenol; spas-  
5 molytics such as aminopromazine, caronerine, difemerine, fencarbamide, tiopramide and moxaverine; testosterone derivates such as testosterone enantate and testosterone-(4-methylpentanoate); tranquilizers such as azaperone and buramate; virustatics such as arildon; vitamin A derivates such as retinol, retinol acetate and retinol palmitate; vitamin E derivates  
10 such as tocopherol acetate, tocopherol succinate and tocopherol nicotinate; menadione; cholecalciferol; insecticides, pesticides and herbicides such as acephate, cyfluthrin, azinphosphomethyl, cypermethrine, substituted phenyl thiophosphates, fenclophos, permethrine, piperonal, tetramethrine and tri-fluraline.

15 As with SLPs, suspensions of PBAs can be stabilized by amphiphatic compounds. Principally the same ionic and nonionic surfactants which may be employed for the stabilization of SLPs are also suitable for the preparation of PBA suspensions. The choice of stabilizing agents depends on the physicochemical properties of both the bioactive substance and the disper-  
20 sion medium as well as on the desired surface characteristics of the particles.

The aqueous phase in which the PBAs are dispersed should contain water-soluble (or dispersable) stabilizers; isotonicity agents such as glycerol or xylitol; cryoprotectants such as sucrose, glucose, trehalose etc; electro-  
25 lytes; buffers; antiflocculants such as sodium citrate, sodium pyrophosphate or sodium dodecylsulfate; preservatives. Although water is the preferred dispersion medium the invention is, however, not restricted to aqueous dispersions alone but can be extended to any other pharmacologically acceptable liquid such as ethanol, propylene glycol and methyl-isobutyl-ketone, or a  
30 mixture thereof.

Depending on the characteristics of the employed stabilizers the coexistence of other colloidal structures such as micelles and vesicles in suspensions of PBAs cannot be ruled out.

Suspensions of PBAs are typically prepared by an emulsification process similar to that of SLPs. The molten drug or bioactive substance or a mixture of such compounds is emulsified in a pharmacologically acceptable liquid immiscible with the melt, preferably by high-pressure homogenization. Emulsification is also possible by sonication, high-speed stirring, vortexing and vigorous hand shaking. The liquid is heated to the temperature of the melt before mixing and may contain for example isotonicity agents, buffering substances, cryoprotectants and/or preservatives.

The stabilizing amphiphatic compounds are added either to the melt and to the liquid or to the liquid only, depending on their physicochemical characteristics. Stabilizers may also be added or exchanged after homogenization, for example by the adsorption of polymers or by dialysis.

The PBAs manufactured according to the above described process can be categorized in two distinguishable groups.

The PBAs of the first group are characterized in that they are water-insoluble at the temperature of emulsion preparation and will not be solubilized by the excess of stabilizers or form micelles by themselves, the particle size of PBAs remaining unchanged before and after cooling to room temperature.

The PBAs of the second group are characterized in that they are partly water-soluble at the temperature of emulsion preparation and/or are able to form mixed micelles by the excess of stabilizers and/or form micelles by themselves, leading to an increase of particle size after cooling to room temperature due to, for example, crystal growth and/or precipitation of dissolved bioactive agent from the supersaturated solution and/or due to mass transport from smaller to larger particles, for example in micelles and/or by processes such as Ostwald ripening.

In a subsequent step the liquid phase can be removed by freeze-drying, for example, producing a reconstitutable powder which can also be processed into other pharmaceutical formulations.

PBAs are finely dispersed particles consisting of a matrix of bioactive material surrounded by one or more layers rich in surfactant. The particle size and the size distribution as well as the particle shape and the surfact-

ant coating depend on the properties and amounts of the matrix forming bioactive substances and the stabilizing agents, the ratio of bioactive material to amphiphatic compounds as well as on the way of emulsification.

## 5 EXAMPLES

### Example 1: Method of preparation of tripalmitate SLPs.

In a thermostated vial 4.0 g tripalmitate (tripalmitin, 99% pure, Fluka) is heated to 75°C to melt the lipid. In the lipid melt 0.48 g soy bean lecithin (Lipoid S 100, Lipoid KG) is dispersed by probe sonication (MSE  
10 Soniprep 150) until the dispersion appears optically clear. 0.16 g sodium glycocholate (glycocholic acid, sodium salt 99%, Sigma) and 4 mg thiomersal (Synopharm) is dissolved in 36 ml bidistilled water. The aqueous phase is heated to 75°C and added to the lipid melt. A crude dispersion is produced by probe sonication for approximately 2 minutes. The crude disper-  
15 sion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 5 times through the homogenizer at a pressure of 500 bar. Homogenization with this equipment is accomplished by extrusion through a small ring-shaped orifice. The homogenized dispersion is allowed to stand at room temperature to cool off. The dispersion  
20 reveals trace amounts of visible fat particles which are separated from the dispersion by filtering it through a 0.45 µm sterile filter.

The importance of the admixture of highly mobile surfactants such as bile salts with regard to the particle size distribution and the stability of SLP dispersions is demonstrated below, e.g. in Examples 2, 5 to 7 and 13 to 15.

25 The mean particle size after preparation (by number) of the tripalmitate SLPs determined by photon correlation spectroscopy (PCS, Malvern Zetasizer 3) is 205 nm. After 15 months of storage the particles show no visible signs of aggregation, creaming, sedimentation or phase separation. A PCS multiangle measurement (Malvern Zetasizer 3, detection at five different angles: 50, 70, 90, 110 and 130 degrees) reveals a monomodal particle size distribution (by number) with a peak at 250 nm (Fig. 5).  
30

At temperatures below the melting point of the lipid matrix tripalmitate SLPs are predominantly anisometrical particles as demonstrated in Fig. 1

which is a transmission electron micrograph of a freeze-fractured specimen of the tripalmitate SLPs of Example 1. Before preparation of the specimen the sample is stored at room temperature for 5 months. The sample is freeze-fractured at 173 K in a freeze-fracture unit BAF 400 (Balzers AG, CH-Liechtenstein). Fast freezing is accomplished by slush into melting propane. Shadowing of the sample is performed with platinum/carbon (layer thickness 2 nm) at 45 degrees and with pure carbon at 90 degrees for replica preparation. Replica are cleaned with a 1:1 (v/v) chloroform/ethanol mixture. Replica on uncoated grids are viewed with an electron microscope EM 300 (Philips, D-Kassel).

In the anisometrical tripalmitate particles the glyceride is present in the stable  $\beta$ -crystalline polymorph as indicated by thermoanalytical investigations. Fig. 2 presents a differential scanning calorimetric (DSC) thermogram of SLPs of Example 1 and of pure tripalmitate. The samples are weighed accurately into standard aluminium pans. Thermograms are recorded from 20°C to 90°C at a scan rate of 10°C/minute on a Perkin Elmer calorimeter DSC-7. The detected transition peaks correspond to the melting of tripalmitate  $\beta$ -crystals. The melting point of tripalmitate SLPs is shifted to a lower temperature compared to that of pure tripalmitate due to the presence of phospholipids and due to the small crystallite size.

Example 2: Method of preparation of isotonic tripalmitate SLPs.

7.0 g tripalmitate (tripalmitin, Fluka) is melted in a vial thermostated at 75°C. 840 mg soy bean lecithin (Lipoid S 100, Lipoid KG) is dispersed in the tripalmitate melt as described in Example 1. The aqueous phase containing 1.575 g glycerol, 280 mg sodium glycocholate and 4 mg thiomersal is heated to 75°C and added to the lipid melt to a weight of 70 g. A crude dispersion is produced by sonication for approximately 2 minutes. The crude dispersion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 10 times through the homogenizer at a pressure of 800 bar. The homogenized dispersion is allowed to stand at room temperature to cool off.

The mean particle size by number of the isotonic tripalmitate SLPs determined by PCS is 125.9 nm after preparation and 116.2 nm after 50 days of storage, i.e. there was practically no particle growth. The slight deviations of the values fall into the range of accuracy of the sizing method.

- 5 The PCS particle size distribution is compared to that of the commercially available lipid emulsion for parenteral nutrition Intralipid™ in Fig. 6. Intralipid™ is composed of 10% soy bean oil, 1.2% fractionated phospholipids and 2.25% glycerol dispersed in water for injection. It can be observed that the particle size distribution of tripalmitate SLPs of Example 2 is significantly smaller and more narrow than that of Intralipid™. In contrast to Example 1 the addition of glycerol results in a noticeable difference in the particle size distribution. Whereas the SLP dispersion of Example 1 contains trace amounts of visible suspension particles after being cooled to room temperature from the hot emulsion no macroscopically visible suspension particles are observed in the dispersion of Example 2.
- 10
- 15

Investigations by synchrotron radiation wide-angle X-ray diffraction (Fig. 3) and differential scanning calorimetry reveal that the tripalmitate in SLPs is present in the stable  $\beta$ -polymorphic form at room temperature.

20 Example 3: Preparation of hard fat SLPs by microfluidization.

- 3.0 g hard fat (Witepsol™ W35, Hüls AG) is melted in a thermostated vial at 75°C. 1.8 g soy bean lecithin (Phospholipon 100, Natterman) is dispersed in the tripalmitate melt as described in Example 1. The aqueous phase containing 375 mg sodium glycocholate, 2.25 g glycerol and 10 mg thiomersal is heated to 75°C and added to the lipid melt to a weight of 100 g. A crude dispersion is produced by ultra-turrax vortexing for approximately 2 minutes. The crude dispersion is transferred to a microfluidizer (Microfluidics Microfluidizer M-110T), a high-pressure homogenizer of the jet-stream principle which is immersed in a thermostated water bath (70°C). The dispersion is cycled through the microfluidizer for 10 minutes and allowed to stand at room temperature to cool off.
- 25
- 30

The mean particle size of hard fat SLPs after preparation is 45.9 nm as determined by PCS.

During homogenization a sample for particle sizing is drawn each minute in order to monitor the time course of homogenization. Fig. 7 displays the mean particle size versus homogenization time. The mean particle diameter is decreasing with time and levels off at the end of homogenization.

Example 4: Long-term stability of hard fat SLPs prepared by microfluidization.

The stability of hard fat SLPs is monitored over a period of one year. During this time the sample is stored in a refrigerator at approximately +4°C. After certain time intervals the particle size distribution of the sample is determined by PCS. Fig. 8 demonstrates that the mean particle size of hard fat SLPs is practically constant over the monitored period of one year.

Example 5: Preparation of unstable SLPs dispersions.

In a thermostated vial 4.0 g tripalmitate (Dynasan 116, Hüls AG) is heated to 75°C to melt the lipid. In the lipid melt 0.48 g soy bean lecithin (Lipoid S 100, Lipoid KG) is dispersed by probe sonication (MSE Soniprep 150) until the dispersion appears optically clear. 4 mg thiomersal and 0.9 g glycerol is dissolved in 35.6 ml bidistilled water. The aqueous phase is heated to 75°C and added to the lipid melt. A crude dispersion is produced by probe sonication for approximately 2 minutes. The crude dispersion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 5 times through the homogenizer at a pressure of 500 bar. The homogenized dispersion is allowed to stand at room temperature to cool off. On storage the SLP dispersion becomes a milky semi-solid, ointment-like gel.

3.0 g hard fat (Witepsol™ W35, Hüls AG) is melted in a thermostated vial at 75°C. 1.8 g soy bean lecithin (Phospholipon 100, Natterman) is dispersed in the tripalmitate melt as described in Example 1. The aqueous phase containing 10 mg thiomersal is heated to 75°C and added to the lipid melt to a weight of 100 g. A crude dispersion is produced by ultraturrax vortexing for approximately 2 minutes. The crude dispersion is

transferred to a microfluidizer (Microfluidics Microfluidizer M-11OT) which is immersed in a thermostated water bath (70°C). The dispersion is cycled through the microfluidizer for 10 minutes and allowed to stand at room temperature to cool off. On storage the SLP dispersion becomes a turbid semi-solid, ointment-like gel. A transmission electron micrograph of this gel is presented in Fig. 4.

In a thermostated vial 4.0 g tripalmitate (Dynasan 116, Hüls AG) is heated to 80°C to melt the lipid. In the lipid melt 0.8 g of a soy bean lecithin mixture (Lipoid S 75, Lipoid KG) is dispersed by probe sonication (MSE Soniprep 150) until the dispersion appears optically clear. 4 mg thiomersal is dissolved in 35.6 ml bidistilled water. The aqueous phase is heated to 80°C and added to the lipid melt. A crude dispersion is produced by probe sonication for approximately 2 minutes. The crude dispersion is transferred to a thermostated high pressure homogenizer (APV Gaulin Micron Lab 40) and passed 5 times through the homogenizer at a pressure of 800 bar. The homogenized dispersion is filled in a glass vial and allowed to stand at room temperature to cool off. On cooling to room temperature the dispersion forms semi-solid looking fat aggregates on the wall of the glass vial. The dispersion gelatinizes when shear forces are applied, for example by passing it through a hypodermic syringe.

Obviously the use of phospholipids only as stabilizers, as found in commercial parenteral oil-in-water emulsions, does not yield stable systems in the case of SLP suspensions. Even the employment of phospholipids such as Lipoid S 75 which induces a considerably high negative net charge cannot provide a sufficient stabilization. Electrostatic repulsion alone cannot be the basic stabilization mechanism of SLPs as will be further outlined in Examples 6, 7 and 13.

Example 6: Preparation of tripalmitate SLPs sterically stabilized by tyloxapol.

A series of tripalmitate SLP dispersions stabilized by tyloxapol (Eastman Kodak) are prepared with varying lipid/surfactant ratios. The SLP dispersions are manufactured according to the following procedure:



Tyloxapol is dissolved in heated bidistilled water while the temperature is held below the cloud point of tyloxapol (approximately 90-95°C). The tyloxapol solution of a temperature of 80°C is added to the molten tripalmitate or, respectively, tripalmitate/lecithin dispersion of the same temperature. A crude emulsion is prepared by probe sonication for approximately 2 minutes. Then the crude emulsion is passed 5 times through a high-pressure homogenizer at a pressure of 1200 bar. The homogenized dispersion is allowed to stand at room temperature to cool off. All dispersions contain 2.5% glycerol and 0.01% thiomersal.

Table 1 gives the composition of the prepared SLP dispersions and their mean particle size after preparation (by number) as determined by PCS. The asterix (\*) in the particle size column indicates that the dispersions display a bimodal size distribution with particle sizes considerably larger than indicated by the mean particle size. It turns out that sterically stabilized SLP dispersions require a high amount of surfactant in order to obtain homogeneously sized SLPs. In case of SLPs stabilized by tyloxapol and phospholipids the ratio of the surfactants needs to be optimized. In the present series a tyloxapol/lecithin ratio of at least 1:1 turned out to yield homogeneously sized SLPs. With increasing ratio the mean particle size is decreasing. As with examples 1 to 3 the addition of a highly mobile surfactant which is able to form micelles is required to obtain stable dispersions. The high amount of surfactant is needed to create a reservoir of surfactant in the dispersion medium that can provide enough surfactant molecules at the moment when the molten lipids recrystallize and form anisometrical particles with a large specific surface area.

**Table 1:** SLP dispersions sterically stabilized by tyloxapol.

Composition (w%)			Mean particle size (nm)
TP	Tyl	PL	
10	2	-	138.0*
10	4	-	84.9
10	0.7	2	487.4*
10	1	2	207.4*
10	2	2	102.8
10	4.5	3	60.9

Abbreviations: TP = tripalmitate, Tyl = tyloxapol, PL = phospholipids (Lipoid S 100).

**Example 7:** Preparation of tripalmitate SLPs sterically stabilized by poloxamers.

1.2 g soy bean lecithin (Lipoid S 100; Lipoid KG) is dispersed in 4.0 g molten tripalmitate (Dynasan 116, Hüls AG) by probe sonication at a temperature of 80°C. 1.8 g poloxamer (Pluronic™ F68, BASF), 0.9 g glycerol and 4 mg thiomersal is dissolved in 32.1 g bidistilled water heated to 80°C. The heated solution is added to the lipid melt and a crude dispersion is prepared by 2 minutes probe sonication. The crude dispersion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 5 times through the homogenizer at a pressure of 1200 bar. The homogenized dispersion is allowed to stand at room temperature to cool off.

The poloxamer stabilized SLPs display a monomodal size distribution with a mean particle size (by number) after preparation of 77.9 nm determined by PCS. Due to the presence of an excess of highly mobile surfactant in the aqueous phase the system is stabilized on recrystallization

of the molten lipids and a gelation as found with systems stabilized by phospholipids only does not occur.

5     **Example 8:** The influence of homogenization pressure on the mean particle size of SLPs.

SLPs of the following composition are prepared at different homogenization pressures. The SLP dispersions are composed of 3% tripalmitate (Dynasan 116, Hüls AG), 1.5% tyloxapol, 1% soy bean lecithin (Lipoid S 100, Lipoid KG), 0.01% thiomersal and bidistilled water to 100% (by weight). The lecithin is dispersed in the molten tripalmitate (80°C) by probe sonication until the dispersion appears optically clear. Tyloxapol is dissolved in warm water (80°C) containing thiomersal. The SLP dispersions are prepared as described in Example 6.

15     Fig. 9 displays the influence of homogenization pressure on the mean particle size of the SLPs. With increasing pressure the particle size is decreasing and the particle size distribution becomes more narrow.

**Example 9:** The influence of homogenization passes on the mean particle size of SLPs.

20     Tripalmitate SLPs composed of 3% tripalmitate (Dynasan 116, Hüls AG), 1.5% tyloxapol, 1% soy bean lecithin (Lipoid S 100, Lipoid KG), 0.01% thiomersal and bidistilled water to 100% (by weight) is prepared at a pressure of 800 bar as described in Example 6. Samples for size measurements are taken from the dispersion after preparation of the crude emulsion and after each pass through the homogenizer.

Fig. 10 presents the influence of the number of homogenization passes on the mean particle size of SLPs which is decreasing with increasing number of passes.

30     **Example 10:** Preparation of SLPs by probe sonication - Influence of sonication time on the mean particle size of SLPs.

In a sonication vial thermostated at 80°C 1.20 g tripalmitate is melted. In the lipid melt 0.40 g soy bean lecithin (Lipoid S 100) is dispersed

by probe sonication until the dispersion appears optically clear. 0.60 g tyloxapol and 4 mg thiomersal is dissolved in bidistilled water heated to 80°C. The aqueous phase is added to the lipid melt and an SLP dispersion is prepared by probe sonication at 80°C. The sonicator operates at 50% of its maximum power. At certain time intervals (1, 5, 10 and 15 minutes) samples are taken from the dispersion for size measurements. After 30 minutes probe sonication is stopped and the dispersion is allowed to stand at room temperature to cool off.

The influence of sonication time on the mean particle size of the SLPs is displayed in Fig. 11. With increasing sonication time the mean particle size is decreasing and the size distribution becomes more narrow.

Example 11: Preparation of SLPs by stirring.

An SLP dispersion composed as in Examples 9 and 10 is prepared by use of a heated magnetic stirrer (Pyro-Magnestir, Lab-Line). The lecithin is dispersed in the tripalmitate as described before. The heated aqueous phase is added to the melt. A dispersion is produced by stirring the mixture for 30 minutes at a temperature of 80°C. The dispersion is allowed to stand at room temperature to cool off.

The mean particle size after preparation (by volume) of the SLP dispersion is 59.5 µm determined by laser diffractometry (Malvern Mastersizer MS20). The maximum particle size measured is 250 µm. In contrast to high pressure homogenization and probe sonication, stirring produces relatively large particles in the micrometer size range.

25

Example 12: Influence of the matrix constituent on the mean particle size of SLPs.

SLP dispersions composed of 10% matrix constituent, 1.2% soy bean lecithin (Lipoid S 100), 0.4% sodium glycocholate, 2.25% glycerol and 0.01% thiomersal in bidistilled water to 100% are prepared as described in Example 1. Five different matrix constituents are employed: the waxes cetylpalmitate and white bees-wax and the triglycerides trilaurate, trimyristate and tripalmitate.

30

Table 2 presents the PCS mean particle sizes of the different SLP dispersions and the melting points of the matrix constituents.

Table 2: Influence of matrix constituents.

Matrix	Melting point (°C)	Mean size of SLPs (nm)
Cetylpalmitate	45.5	141.0
White bees-wax	62.5	195.3
Trilaurate	45.0	137.2
Trimyristate	56.5	161.1
Tripalmitate	63.0	209.2

The mean particle size of SLPs is increasing with the melting point of the matrix constituent.

Example 13: Influence of emulsifier type and amount on the mean particle size and stability of SLPs.

Tripalmitate SLP dispersions with different types and amounts of emulsifiers are prepared as described in Example 2. The composition of the different batches is given in Table 3. All dispersions contain 2.25% glycerol and 0.01% thiomersal.

The mean particle size of the different batches of SLPs is presented in Fig. 12. The mean particle size depends on the type and amount of emulsifier.

**Table 3:** Compositions of SLP batches (in w%).

Batch no	TP	PL	SGC	Plu
1	10%	1.2%	-	-
2	10%	1.2%	0.4%	-
3	10%	2.4%	0.4%	-
4	10%	-	-	1.8%
5	10%	-	-	3.6%

- Abbreviations: TP = tripalmitate, PL = phospholipids (Lipoid S 100), SGC = sodium glycocholate, Plu = Pluronic F68.

The combination of phospholipids and bile salts is most efficient with regard to the mean particle size and the stability. The system stabilized by phospholipids only gelatinizes and forms an ointment-like semi-solid gel on storage. The systems stabilized by Pluronic F68 tend to gelatinize when shear forces are applied, i.e. when the particles are forced to get closer to each other. Obviously the steric stabilization by poloxamers is not sufficient in this case. As a result the optimum stabilization is that by a surfactant combination of emulsifiers that are present in and act from the lipid side (such as phospholipids) and of emulsifiers that constitute a reservoir of highly mobile surfactant molecules in the dispersion medium (such as bile salts, tyloxapol and poloxamers). Though repulsion forces represent an important factor for the long-term stability, the basic mechanism of SLP stabilization is the high mobility of the excess of surfactant which provides for the immediate surface coverage of newly created surfaces during recrystallization of the molten lipids.

**Example 14:** Effect of bile salt as co-emulsifier of phospholipid stabilized SLPs.

Phospholipid-stabilized SLP dispersions employing different matrices (tripalmitate, hard fat) are prepared with or without the addition of bile salt (sodium glycocholate) to the aqueous phase according to the method described in Example 1. All dispersions contain 2.25% glycerol and 0.01% thiomersal. Emulsification of the crude dispersions is performed by high pressure homogenization (APV Gaulin Micron Lab 40) under different homogenization conditions. The following dispersions were prepared:

Composition	Homogenization conditions
7.0 g TP, 0.84 g PL, 62.2 g H <sub>2</sub> O	3 x 500 bar
7.0 g TP, 0.84 g PL, 0.28 g BS, 61.9 g H <sub>2</sub> O	3 x 500 bar
7.0 g TP, 0.84 g PL, 62.2 g H <sub>2</sub> O	10 x 1200 bar
7.0 g TP, 0.84 g PL, 0.28 g BS, 61.9 g H <sub>2</sub> O	10 x 1200 bar
7.0 g HF, 0.84 g PL, 62.2 g H <sub>2</sub> O	3 x 500 bar
7.0 g HF, 0.84 g PL, 0.28 g BS, 61.9 g H <sub>2</sub> O	3 x 500 bar
7.0 g HF, 0.84 g PL, 62.2 g H <sub>2</sub> O	10 x 1200 bar
7.0 g HF, 0.84 g PL, 0.28 g BS, 61.9 g H <sub>2</sub> O	10 x 1200 bar

Abbreviations: BS = bile salt; H<sub>2</sub>O = bidistilled water, HF = hard fat (Witepsol W35); PL = phospholipids (Lipoid S 100); TP = tripalmitate.

The mean particle size of the dispersions as determined by PCS after preparation is presented in Fig. 13. This example demonstrates the effect of bile salts as co-emulsifier in the aqueous phase on the particle size of phospholipid stabilized SLPs. It is clearly shown that the addition of bile salts reduces the mean particle size of SLPs by up to 57%. Thus, by the use of bile salts as co-emulsifier extremely fine dispersions can be obtained. The effect of the bile salt can be attributed to the high mobility of this micelle forming ionic surfactant which enables the surfactant molecules to immediately cover freshly generated surfaces during the homogenization process. The phospholipids which tend to form liquid crystalline structures in

the aqueous phase are not sufficiently mobile to provide the immediate stabilization of freshly created particles so that instantaneous coalescence occurs in case there is no highly mobile co-surfactant in the aqueous phase.

5

Example 15: Preparation of trimyristate SLPs stabilized by a lecithin/bile salt blend.

In a thermostated vial 7.0 g trimyristate (Dynasan 114, Hüls AG) is melted at 70°C. 0.96 g phospholipids (Lipoid S 100) are dispersed in the melt by probe sonication. A solution of 280 mg sodium glycocholate, 1.6 g glycerol and 7 mg thiomersal in 61 ml bidistilled water is heated to 70°C and added to the melt. A crude dispersion is prepared by probe sonication for approximately 2 minutes. The crude dispersion is transferred to a high pressure homogenizer (APV Gaulin Micron Lab 40) thermostated at approximately 90°C and is passed 5 times through the homogenizer at a pressure of 500 bar. The homogenized dispersion is allowed to stand at room temperature to cool off.

10

15

20

25

The mean particle size after preparation determined by PCS is 155.7 nm. In laser diffractometry (Malvern Mastersizer MS20) no particles above 1 µm can be detected. The particle size distribution derived from laser diffractometry is presented in Fig. 14. This example demonstrates that the use of bile salts as co-emulsifier of phospholipid stabilized SLPs efficiently prevents the formation of particles larger than 1 µm due to the rapid coverage of freshly created surfaces during homogenization, thereby minimizing immediate coalescence.

Example 16: Preparation of tripalmitate SLPs without ultrasonication (method A).

In a thermostated vial 4.0 g tripalmitate (Dynasan 116, Hüls AG) is melted at 85°C. 0.96 g lecithin (Lipoid S 100) is dissolved in ethanol. The lecithin solution is added to the melt. The ethanol is evaporated at a temperature of 85°C. 160 mg sodium glycocholate, 0.9 g glycerol and 4 mg thiomersal is dissolved in 35 ml bidistilled water. The solution is heated to

30



85°C and added to the melt. A crude dispersion is prepared by ultra-turrax vortexing for approximately 2 minutes. The crude dispersion is transferred to a high-pressure homogenizer (APV Gaulin Micron Lab 40) thermostated at approximately 90°C and passed 10 times through the homogenizer at a pressure of 800 bar. The homogenized dispersion is allowed to stand at room temperature to cool off.

Example 17: Preparation of tripalmitate SLPs without ultrasonication (method B).

In a thermostated vial 4.0 g tripalmitate (Dynasan 116, Hüls AG) is melted at 85°C. 0.96 g lecithin (Lipoid S 100) is added to the melt. The mixture is shaken until the lecithin is completely dispersed in the melt and the dispersion appears isotropic. 160 mg sodium glycocholate, 0.9 g glycerol and 4 mg thiomersal is dissolved in 35 ml bidistilled water. The solution is heated to 85°C and is added to the melt. A crude dispersion is prepared by ultra-turrax vortexing for approximately 2 minutes. The crude dispersion is transferred to a high-pressure homogenizer (APV Gaulin Micron Lab 40) thermostated at approximately 90°C and passed 10 times through the homogenizer at a pressure of 800 bar. The homogenized dispersion is allowed to stand at room temperature to cool off.

Example 18: Preparation of tripalmitate SLPs by dispersing phospholipids in the aqueous phase.

In a thermostated vial 4.0 g tripalmitate (Dynasan 116) is melted at 80°C. 0.96 g phospholipids (Lipoid S 100) is dispersed in 35 ml of an aqueous solution of 160 mg sodium glycocholate, 0.9 g glycerol and 4 mg thiomersal by stirring for approximately one hour. The phospholipid dispersion is heated to 80°C and added to the tripalmitate melt. A crude dispersion is prepared by probe sonication for approximately 2 minutes. The crude dispersion is transferred to a high-pressure homogenizer (APV Gaulin Micron Lab 40) thermostated at approximately 90°C and passed 10 times through the homogenizer at a pressure of 800 bar. The homogenized dispersion is allowed to stand at room temperature to cool off.

Example 19: Preparation of tripalmitate SLPs stabilized by a highly mobile surfactant.

In a thermostated vial 5.0 g tripalmitate is melted at 80°C. 600 mg sodium glycocholate is dissolved in 44.4 g bidistilled water containing 1.13 g glycerol and 0.01% thiomersal. The aqueous solution is heated to 80°C and added to the melt. A crude dispersion is prepared by sonication for approximately 5 minutes. The crude dispersion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 8 times through the homogenizer at a pressure of 800 bar. The dispersion is allowed to stand at room temperature to cool off.

The mean particle size (by number) of the SLP dispersion after preparation is 96.8 nm determined by PCS. The size distribution is narrow and monomodal.

This example demonstrates that it is possible to prepare small homogeneously sized SLPs by the use of one surfactant only, such as the bile salt sodium glycocholate, provided the surfactant is highly mobile and constitutes a reservoir of stabilizer in the aqueous phase in order to provide for the stabilization of newly created surfaces during recrystallization of the SLP matrix.

Example 20: Long-term stability of different SLP dispersions.

Several different SLP dispersions are prepared according to the method described in Example 2. All dispersions contain 2.25 % glycerol as isotonicity agent and 0.01 % thiomersal as a preservative. The long-term stability of the dispersions is judged from repeated size measurements (by PCS) over a period of 18 months. The dispersions are stored at refrigeration temperatures. For comparison a soy bean oil emulsion system is included. The composition of the dispersions and their mean particle sizes during storage are summarized in Table 4.

Table 4

Composition (w %)			Mean particle size (nm) after	
Matrix	PI	SGC	Preparation	18 months
10% TP	1.2%	0.4%	125.9	121.6
10% TP	2.4%	0.4%	104.5	111.2
10% TP	2.4%	0.4%	103.6	104.7
9.5% TP <sup>1)</sup>				
0.5% GMS	2.4%	0.4%	102.4	102.4
10% SO	2.4%	0.4%	129.6	139.9

Abbreviations: PL = phospholipid (Lipoid S 100); SGC = sodium glyco-  
cholate; TP = tripalmitate; GMS = glycerol monostearate; SO = soy bean  
oil.

<sup>1)</sup> The SLP dispersion contains 5 % (related to fat phase) of the cardio-  
protective drug ubidecarenone.

It is shown that the mean particle size of the dispersions remains  
practically unchanged during storage for 18 months. Thus, the results  
demonstrate that drug-free and drug-loaded SLP dispersions exhibit a long-  
term stability similar to that of lipid emulsions.

#### Example 21: Sterile filtration of tripalmitate SLPs.

40 ml of a crude SLP dispersion composed of 3% tripalmitate  
(Dynasan 116, Hüls AG), 1.5% tyloxapol, 1% lecithin (Lipoid S 100), 2.25%  
glycerol and 0.01% thiomersal in bidistilled water to 100% is prepared  
according to the method described in Example 6. The crude dispersion is  
passed 5 times through a thermostated homogenizer (APV Micron Lab 40)  
at a pressure of 1200 bar. Half the volume of the batch is allowed to stand  
at room temperature to cool off, whereas the rest is filtered through a sterile

syringe filter (Nalgene SFCA, 0.2  $\mu$ m pore size) before being cooled to the recrystallization temperature of the molten lipids.

The particle size distributon of both samples is determined by PCS. The mean particle size of the unfiltered sample is 56.7 nm and that of the sterile filtered SLP dispersion is 53.2 nm, i e both samples have practically the same mean particle size.

Example 22: Sterilization of tripalmitate SLPs by autoclaving.

40 ml of a crude SLP dispersion composed of 3% tripalmitate (Dynasan 116, Hüls AG), 1.8% lecithin (Lipoid S 100), 0.6% sodium glycocholate, 2.25% glycerol and 0.01% thiomersal in bidistilled water to 100% is prepared according to the method described in Example 2. The crude dispersion is passed 10 times through a thermostated homogenizer (APV Micron Lab 40) at a pressure of 1200 bar.

Before being cooled to the recrystallization temperature of the molten lipids the SLP dispersion is filled into an injection vial and sterilized by autoclaving at 121°C/2 atm for 45 minutes. The autoclaved dispersion is allowed to stand at room temperature to cool off. It shows no signs of aggregation or phase separation and has a mean particle size of 65.9 nm determined by PCS.

Example 23: Lyophilization of SLPs.

In a thermostated vial 3.5 g tripalmitate (Dynasan 116, Hüls AG) is melted at 75°C, and 1.05 g lecithin (Lipoid S 100, Lipoid KG) is dispersed in the melt by probe sonication. 1.58 g tyloxapol, 14 g sucrose and 7 mg thiomersal is dissolved in 50 ml distilled water heated to 75°C and the aqueous phase is added to the lipid melt. A crude dispersion is prepared by probe sonication and then passed 5 times through a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) at a pressure of 900 bar. The homogenized dispersion is passed through a 0.2  $\mu$ m sterile filter.

For lyophilization the dispersion is filled into plane-bottom vials which are immersed in liquid nitrogen for 1 minute and transferred to the freeze-

drying chamber. Samples are freeze-dried for 36 hours under vacuum at a recipient temperature of -40°C.

Freeze-drying yields easily redispersible fine powders. The particle size of the SLP dispersions is determined by PCS prior to lyophilization and after reconstitution of the lyophilized powders with distilled water. The mean particle size prior to lyophilization is 79 nm and that of the reconstituted dispersion is 87 nm, i.e. there is practically no change in mean particle size after lyophilization.

10 Example 24: Surface modification by adsorption of polymers.

In a thermostalized vial 4.0 g tripalmitate (Dynasan 116, Hüls AG) is melted at 80°C and 1.6 g soy bean lecithin (Lipoid S 100) is dispersed in the melt by probe sonication. 35.25 g of an aqueous solution of 0.01% thiomersal is heated to 80°C and added to the melt. A crude dispersion is produced by probe sonication which is then passed five times through a high-pressure homogenizer at a pressure of 1200 bar. The dispersion is filtered through a 0.2 µm syringe filter. The batch is divided into 3 parts of equal volumes. One part is diluted with the same amount of water and stored at 90°C to prevent gelation of the phospholipid stabilized dispersion on cooling down below the recrystallization temperature. The other two parts of the batch are incubated overnight with equal volumes of 6% (w/w) poloxamer 407 (Pluronic F127, BASF) and 6 % (w/w) poloxamine 908 (Tetronic 908, BASF) solution, respectively, in such a way that the polymer solution is added to the SLP dispersion prior to being cooled below the recrystallization temperature of SLPs to provide for the immediate availability of polymer molecules as soon as new surfaces are created due to recrystallization. Both polymers have been described in literature to modify the bio-distribution of intravenously administered colloidal particles.

The modification of the surface properties of tripalmitate SLPs is demonstrated by differences in the zeta potential. Zeta potentials were determined by laser Doppler anemometry in a microelectrophoresis cell (Malvern Zetasizer 3). The results are summarized in Table 5.

Table 5: Zetapotential of surface modified SLPs.

Composition [% (w/w)]			Zetapotential [mV]
TP	PL	Polymer	
5%	2%	-	-29.6
5%	2%	3% F127	-1.9
5%	2%	3% T908	-2.9

Abbreviations: TP = tripalmitate, PL = phospholipids, F127 = pluronic F127,  
T908 = Tetronic 908.

The incubation of SLPs with block copolymers of the poloxamer and poloxamine type results in a decrease of the zetapotential. Due to the adsorption of the polymers the surfaces become more hydrophobic. The hydrophobicity of the surface is described to be one of the factors governing the RES (reticuloendothelial system) activity and the biodistribution of colloidal particles.

Example 25: Preparation of SLPs loaded with the cardio-protective drug ubidecarenone.

Three different types of SLPs containing the cardio-protective drug ubidecarenone are prepared. The SLPs are composed as summarized in table 6. All dispersions contain 2.25% glycerol and 0.01% thiomersal.

Batch 1 and 2 are prepared by dispersing lecithin in the molten matrix constituent as described before. In this melt ubidecarenone is dissolved. After addition of the aqueous phase containing sodium glycolate, glycerol and thiomersal a crude dispersion is prepared by probe sonication. It is transferred to a thermostated homogenizer (APV Micron Lab 40) and passed through the homogenizer ten times at a pressure of 800 bar. The dispersions are allowed to stand at room temperature to cool off.

Batch 3 is prepared by dispersing lecithin in the molten matrix constituent as described before. In this melt ubidecarenone is dissolved. After addition of the aqueous phase containing tyloxapol, glycerol and thiomersal, a crude dispersion is prepared by probe sonication. It is transferred to a thermostated homogenizer (APV Micron Lab 40) and passed 5 times through the homogenizer at a pressure of 1200 bar. The dispersion is allowed to stand at room temperature to cool off.

Table 6: Ubidecarenone-loaded SLPs.

Batch no	Composition	Mean particle size
1	10% TP, 2.4% PL, 0.4% SGC, 1% Ubi	80.2 nm
2	10% HF, 1.2% PL, 0.4% SGC, 1% Ubi	78.9 nm
3	3% TP, 1.5% Tyl, 1% PL, 0.2% Ubi	46.8 nm

Abbreviations: TP = tripalmitate, PL = phospholipids, SGC = sodium glycocholate, Ubi = Ubidecarenone, HF = hard fat (Witepsol W35), Tyl = Tyloxapol.

Example 26: Preparation of SLPs loaded with oxazepam.

In a thermostated vial 7.0 g tripalmitate is melted at 80°C. 1.68 g lecithin and 140 mg oxazepam is dispersed in the melt by probe sonication. 60 ml of heated aqueous phase containing 280 mg sodium glycocholate, 1.58 g glycerol and 7 mg thiomersal is added to the melt and a crude dispersion is prepared by probe sonication. The crude dispersion is homogenized by passing 10 times through a thermostated high-pressure homogenizer at a pressure of 800 bar. The dispersion is allowed to stand at room temperature to cool off. The dispersion of oxazepam loaded SLPs has a mean particle size of 122.7 nm after preparation.

**Example 27:** Preparation and long-term stability of SLPs loaded with diazepam.

In a thermostated vial 4.0 g tripalmitate is melted at 80°C. 0.96 g lecithin and 120 mg diazepam is dispersed in the melt by probe sonication. 35 ml of heated aqueous phase containing 160 mg sodium glycocholate, 0.9 g glycerol and 4 mg thiomersal is added to the melt and a crude dispersion is prepared by probe sonication. The crude dispersion is homogenized by passing 10 times through a thermostated high-pressure homogenizer at a pressure of 800 bar. The dispersion is allowed to stand at room temperature to cool off.

The dispersion of diazepam-loaded SLPs has a mean particle size after preparation of 104.6 nm. After 12 months of storage the mean particle size determined by PCS is 113.9 nm. Precipitation of drug substance during storage is not detected macroscopically. Investigations of the dispersion by polarized light microscopy over the monitored period of 12 months do not reveal the presence of drug crystals.

**Example 28:** Preparation of SLPs loaded with lidocaine.

In a thermostated vial 4.0 g tripalmitate is melted at 80°C. 0.96 g lecithin and 60 mg lidocaine is dispersed in the melt by probe sonication. 35 ml of heated aqueous phase containing 320 mg sodium glycocholate, 0.9 g glycerol and 4 mg thiomersal is added to the melt and a crude dispersion is prepared by probe sonication. The crude dispersion is homogenized by passing 10 times through a thermostated high-pressure homogenizer at a pressure of 800 bar. The dispersion is allowed to stand at room temperature to cool off.

The dispersion of lidocaine loaded SLPs has a mean particle size after preparation of 90.4 nm.

**Example 29:** Preparation and long-term stability of SLPs loaded with prednisolone.

In a thermostated vial 4.0 g tripalmitate is melted at 80°C. 0.48 g lecithin and 80 mg prednisolone is dispersed in the melt by probe sonica-



tion. 36 ml of heated aqueous phase containing 160 mg sodium glyco-  
cholate, 0.9 g glycerol and 4 mg thiomersal is added to the melt and a  
crude dispersion is prepared by probe sonication. The crude dispersion is  
homogenized by passing 10 times through a thermostated high-pressure  
5 homogenizer at a pressure of 800 bar. The dispersion is allowed to stand at  
room temperature to cool off.

The dispersion of prednisolone-loaded SLPs has a mean particle size  
after preparation of 118.3 nm. After 12 months of storage the mean particle  
size determined by PCS is 124.2 nm. Precipitation of drug substance during  
10 storage is not detected macroscopically. Investigations of the dispersion by  
polarized light microscopy over the monitored period of 12 months do not  
reveal the presence of drug crystals.

Example 30: Preparation of SLPs loaded with cortisone.

15 Four different types of SLPs containing cortisone are prepared. The  
SLPs are composed as summarized in table 7. All dispersions contain  
2.25% glycerol and 0.01 thiomersal.

Batch 1 and 2 are prepared by dispersing lecithin in the molten  
matrix constituent as described before. In this melt cortisone is dissolved.  
20 After addition of the aqueous phase containing sodium glycocholate, glyce-  
rol and thiomersal, a crude dispersion is prepared by probe sonication. It is  
transferred to a thermostated homogenizer (APV Micron Lab 40) and  
passed 10 times through the homogenizer. The dispersions are allowed to  
stand at room temperature to cool off.

25 Batch 3 is prepared by dispersing lecithin in the molten matrix  
constituent as described before. In this melt cortisone is dissolved. After  
addition of the aqueous phase containing poloxamer (Pluronic F68), glycerol  
and thiomersal, a crude dispersion is prepared by probe sonication. It is  
transferred to a thermostated homogenizer (APV Micron Lab 40) and  
30 passed 5 times through the homogenizer at a pressure of 1200 bar. The  
dispersion is allowed to stand at room temperature to cool off.

Batch 4 is prepared by dispersing lecithin in the molten matrix con-  
stituent as described before. In this melt cortisone is dissolved. After addi-

tion of the aqueous phase containing tyloxapol, glycerol and thiomersal a crude dispersion is prepared by probe sonication. It is transferred to a thermostated homogenizer (APV Micron Lab 40) and passed through the homogenizer five times at a pressure of 1200 bar. The dispersion is allowed to stand at room temperature to cool off.

**Table 7:** Cortisone-loaded tripalmitate SLPs.

Batch no	Composition	Mean particle size
1	10% TP, 1.2% PL, 0.4% SGC, 0.2% Cort	124.2 nm
2	3% TP, 1.8% PL, 0.6% SGC, 0.3% Cort	67.3 nm
3	10% TP, 4.5% Plu, 3% PL, 0.1% Cort	70.5 nm
4	3% TP, 1.5% Tyl, 1% PL, 0.1% Cort	48.5 nm

Abbreviations: TP = tripalmitate, PL = phospholipids, SGC = sodium glycocholate, Cort = Cortisone, Plu = Pluronic F68, Tyl = Tyloxapol.

**Example 31:** Tripalmitate SLPs loaded with retinol (vitamin A).

In a thermostated vial 1.0 g tripalmitate (Dynasan 116, Hüls AG) is melted at 80°C. 60 mg retinol (vitamin A-alcohol >99 %, Fluka) is dissolved in the melt. 300 mg soy bean lecithin (Lipoid S 100) is dispersed in the melt by probe sonication until the dispersion appears optically clear. 450 mg poloxamer 407 (Pluronic™ F127, BASF) is dissolved in 29.0 g bidistilled water. The aqueous phase is heated to 80°C and added to the melt. A fine dispersion is prepared by probe sonication for 20 minutes. The dispersion is filtered through a 0.2 µm syringe filter to remove metal shed from the ultrasound probe. The dispersion is allowed to stand at room temperature to cool off.

The mean particle size by number after preparation of vitamin A-loaded tripalmitate SLPs is 98.5 nm determined by PCS.

Example 32: Tripalmitate SLPs loaded with phytylmenadione (vitamin K<sub>3</sub>).

In a thermostated vial 1.0 g tripalmitate (Dynasan 116, Hüls AG) is melted at 80°C. 60 mg phytylmenadione (vitamin K<sub>3</sub>, Sigma) and 300 mg soy bean lecithin (Lipoid S 100) is dispersed in the melt by probe sonication until the dispersion appears optically clear. 450 mg poloxamer 407 (Pluronic™ F127, BASF) is dissolved in 28.7 g bidistilled water. The aqueous phase is heated to 80°C and added to the melt. A fine dispersion is prepared by probe sonication for 20 minutes. The dispersion is filtered through a 0.2 µm syringe filter to remove metal shed from the ultrasound probe. The dispersion is allowed to stand at room temperature to cool off. The mean particle size by number after preparation of vitamin K<sub>3</sub>-loaded tripalmitate SLPs is 86.8 nm determined by PCS.

Example 33: Preparation of tripalmitate SLPs loaded with estramustine.

In a thermostated vial 7.0 g tripalmitate (Dynasan 116, Hüls AG) is melted at 80°C. In the melt 1.68 g soy bean lecithin (Lipoid S 100) is dispersed by probe sonication until the dispersion appears optically clear. 40 mg estramustine is dissolved in the tripalmitate/lecithin dispersion. 0.42 g sodium glycocholate and 1.58 g glycerol is dissolved in 60 g bidistilled water. The aqueous phase is heated to 80°C and added to the melt. A crude emulsion is prepared by probe sonication for approximately 2 minutes. The crude emulsion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 10 times through the homogenizer at a pressure of 800 bar. The dispersion is allowed to stand at room temperature to cool off.

Example 34: Physical state of different SLPs at body temperature.

Two batches of SLPs from different matrix constituents are prepared according to the method described in Example 2. Batch 1 is composed of 10% tripalmitate, 0.5% ubidecarenone, 1.2% soy bean lecithin (Lipoid S 100, Lipoid KG), 0.4% sodium glycocholate, 2.25% glycerol, 0.01% thiomersal and bidistilled water to 100% (by weight). Batch 2 is composed of 10% hard fat (Witepsol™ W35, Hüls AG), 0.5% ubidecarenone, 1.2% soy

bean lecithin (Lipoid S 100, Lipoid KG), 0.4% sodium glycocholate, 2.25% glycerol, 0.01% thiomersal and bidistilled water to 100% (by weight).

The physical state of the matrix constituents is determined by synchrotron radiation X-ray diffraction at 20°C and at 38°C. The samples  
5 are placed in thermostated sample holders. The diffraction patterns are recorded for 180 seconds each. Fig. 15a demonstrates that at room temperature (20°C) both batches of SLPs are crystalline. The spacings correspond to the  $\alpha$ -crystalline polymorphs. At body temperature (38°C) the tri-palmitate SLPs are still crystalline whereas no reflections can be detected  
10 for the hard fat SLPs, i.e. they are amorphous and molten (Fig. 15b). The different physical states of these SLPs at body temperature give rise to a different biopharmaceutical behaviour with respect to the release of incorporated drugs or bioactive agents. SLPs molten at body temperature display basically the release characteristics typical of conventional lipid emulsions.  
15 Due to the free diffusion of drug molecules in the liquid lipid the drug can be released from the vehicle relatively fast. In contrast, SLPs which are solid at body temperature give rise to sustained release of incorporated drugs. Since the drug molecules are immobilized in the solid matrix drug release is not diffusion-controlled but depends on the degradation of the  
20 solid lipid matrix in the body and is therefore delayed.

Example 35: Preparation of PBAs from miconazole.

In a thermostated vial 0.4 g miconazole is melted at 90°C. 0.24 g lecithin (Lipoid S 100) is dispersed in the melt by probe sonication until the  
25 dispersion appears optically clear. 0.9 g glycerol, 80 mg sodium glycocholate and 4 mg thiomersal is dissolved in 38.5 ml bidistilled water and heated to 90°C. The aqueous phase is added to the miconazole/lecithin melt and a crude dispersion is produced by probe sonication for 5 minutes. The crude dispersion is transferred to a thermostated high-pressure  
30 homogenizer (APV Gaulin Micron Lab 40) and passed 10 times through the homogenizer at a pressure of 800 bar. The PBA dispersion is allowed to stand at room temperature to cool off.

On cooling the molten miconazole recrystallizes and forms a suspension of miconazole microparticles. The mean particle size (by volume) of miconazole PBAs is 21.8  $\mu\text{m}$  determined by laser diffractometry. The sediment of miconazole PBAs is easily redispersible by slight agitation.

5

Example 36: Preparation of PBAs from ibuprofen.

In a thermostated vial 1.2 g ibuprofen is melted at 85°C. 0.72 g lecithin (Lipoid S 100) is dispersed in the melt by probe sonication until the dispersion appears optically clear. 0.9 g glycerol, 240 mg sodium glyco-  
10 cholate and 4 mg thiomersal is dissolved in 37 ml bidistilled water and heated to 85°C. The aqueous phase is added to the ibuprofen/lecithin melt and a crude dispersion is produced by probe sonication for 5 minutes. The crude dispersion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 6 times through the  
15 homogenizer at a pressure of 800 bar. The PBA dispersion is allowed to stand at room temperature to cool off.

On cooling the molten ibuprofen recrystallizes and forms a suspension of ibuprofen microparticles. The mean particle size (by volume) of ibuprofen PBAs is 61.4  $\mu\text{m}$  determined by laser diffractometry. The sedi-  
20 ment of ibuprofen PBAs is easily redispersible by slight agitation.

Example 37: Dissolution speed of ibuprofen PBAs.

The dissolution speed of ibuprofen PBAs of Example 36 is measured in a laser diffractometer (Malvern Mastersizer MS20) by monitoring the  
25 decay of the so-called obscuration over a period of 10 minutes. The obscuration is a measure of the reduced intensity of unscattered laser light by a sample and is related to the concentration of particles in the laser beam. In parallel the particle size can be measured. For measurement the ibuprofen PBA sample is diluted with water and dispersed by magnetic stirring in  
30 a measuring cell placed in the laser beam line. Fig. 16 presents the decay of obscuration and particle size of a sample of ibuprofen PBAs. Within 10 minutes the obscuration has decayed to zero, i.e. there is no detectable amount of particles hinting at the complete dissolution of the PBAs. The

dissolution of the untreated raw substance ibuprofen cannot be measured by this technique since the substance is only poorly wettable in water.

Example 38: Preparation of PBAs from lidocaine.

5           In a thermostated vial 1.2 g lidocaine is melted at 80°C. 1.2 g  
tyloxapol is dissolved in 37.6 ml bidistilled water and heated to 80°C. The  
aqueous phase is added to the lidocaine melt and a crude dispersion is  
produced by probe sonication for 2 minutes. The crude dispersion is trans-  
ferred to a thermostated high-pressure homogenizer (APV Gaulin Micron  
10 Lab 40) and passed 5 times through the homogenizer at a pressure of 1200  
bar. The PBA dispersion is allowed to stand at room temperature to cool  
off.

On cooling the molten lidocaine recrystallizes into fine needles and  
forms a suspension of lidocaine microparticles. Fig. 17 shows a polarized  
15 microscopic picture of the suspended lidocaine needles. The particle shape  
of the raw material lidocaine-base (Synopharm) is different from that of  
lidocaine PBAs as demonstrated by the polarized microscopic picture of  
Fig. 18.

The mean particle size (by volume) of lidocaine PBAs is 174.2 µm  
20 determined by laser diffractometry. The maximum detected particle size is  
400 µm. The sediment of lidocaine PBAs is easily redispersible by slight  
agitation. The addition of water to PBAs leads to the rapid dissolution of the  
particles. In contrast the raw material lidocaine is only sparingly soluble in  
water and the dissolution speed is much slower. The high dissolution speed  
25 of lidocaine PBAs is a consequence of the modified surface properties and  
the finely dispersed state of the particles. Due to the rapid dissolution a  
determination of the dissolution speed according to the method described in  
Example 37 is not possible.

30   Example 39: Preparation of PBAs from cholecalciferol (vitamin D<sub>3</sub>).

In a thermostated vial 0.8 g cholecalciferol is melted at 95°C. 120  
mg soy bean lecithin (Lipoid S 100) is dispersed in the melt by probe soni-  
cation until the dispersion appears optically clear. 40 mg sodium glycochol-

ate and 0.9 g glycerol is dissolved in 37.92 ml bidistilled water and heated to 95°C. The aqueous phase is added to the cholecalciferol/lecithin dispersion and a crude dispersion is produced by probe sonication for 5 minutes. The crude dispersion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 8 times through the homogenizer at a pressure of 1200 bar. The PBA dispersion is allowed to stand at room temperature to cool off.

The mean particle size after preparation by number of cholecalciferol PBAs is 325.1 nm determined by PCS.

10

Example 40: Preparation of PBAs from estramustine.

In a thermostated vial 2 g estramustine is melted at 105°C. In the melt 0.8 g soy bean lecithin (Lipold S 100) is dispersed by probe sonication until the dispersion appears optically clear. 0.2 g sodium glycocholate and 0.9 g glycerol is dissolved in 36.1 g bidistilled water. The aqueous phase is heated to 95°C and added to the melt. A crude emulsion is prepared by probe sonication for approximately 5 minutes. The crude emulsion is transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and passed 5 times through the homogenizer at a pressure of 1200 bar. The dispersion is allowed to stand at room temperature to cool off.

20

## LEGENDS OF FIGURES

- Fig. 1 Transmission electron micrograph of tripalmitate SLPs of Example 1 after 5 months of storage at room temperature. The bar represents 400 nm.
- 5 Fig. 2 Differential scanning calorimetric (DSC) thermogram of a) pure tripalmitate and of b) tripalmitate SLPs of Example 1. The transition peaks correspond to the melting of the  $\beta$ -crystalline polymorph.
- 10 Fig. 3 Synchrotron radiation wide angle X-ray diffraction pattern of tripalmitate SLPs of Example 2. The reflexions correspond to the  $\beta$ -crystalline polymorph.
- Fig. 4 Transmission electron micrograph of unstable hard fat SLPs of Example 5. The SLP dispersion gelatinized on storage by forming a three-dimensional network. The bar corresponds to 1000 nm.
- 15 Fig. 5 Particle size distribution of tripalmitate SLPs of Example 1 after 15 months of storage. The graph represents the result of a multiangle PCS measurement.
- 20 Fig. 6 PCS particle size distribution of a 10% tripalmitate SLP dispersion compared to that of the commercial lipid emulsion Intralipid® 10%.
- Fig. 7 Influence of microfluidization time on the mean particle size of hard fat SLPs of Example 3.
- 25 Fig. 8 Stability on storage of hard fat SLPs of Example 3 as indicated by the development of the mean particle size with storage time (monitored period: 12 months).
- Fig. 9 Influence of homogenization pressure on the mean particle size of tripalmitate SLPs.
- 30 Fig. 10 Influence of the number of homogenization passes on the mean particle size of tripalmitate SLPs (no 0 corresponds to the crude dispersion prepared by sonication).



- Fig. 11 Influence of sonication time on the mean particle size of tri-  
palmitate SLPs prepared by probe sonication.
- Fig. 12 Influence of type and amount of emulsifier on the mean par-  
ticle size of tripalmitate SLPs prepared according to Example  
5 13.
- Fig. 13 Effect of bile salts as co-emulsifier on the mean particle size  
of different phospholipid stabilized SLP dispersions of Ex-  
ample 14.
- Fig. 14 Particle size distribution of trimyrystate SLPs of Example 15  
as determined by laser diffractometry.  
10
- Fig. 15 The physical state of different drug-loaded SLPs a) at 20°C  
and b) at 38°C determined by synchrotron radiation wide-angle  
X-ray diffraction.
- Fig. 16 Dissolution speed of ibuprofen PBAs of Example 37.
- 15 Fig. 17 Polarized microscopic picture of lidocaine PBAs of Example  
38 (magnification: 150 x).
- Fig. 18 Polarized microscopic picture of lidocaine raw material em-  
ployed for the production of lidocaine PBAs (magnification:  
150 x).

## CLAIMS

1. A process for emulsifying insoluble or sparingly water-soluble agents which are solid at room temperature, c h a r a c t e r i z e d in that  
5 the following steps are carried out:
- a. the solid agent or a mixture of solid agents is melted,
  - b. a dispersion medium is heated to approximately the same temperature as the molten solid agent or the mixture of solid agents,
  - 10 c. one or more highly mobile water-soluble or dispersible stabilizer/stabilizers which does/do not form a separate phase in the dispersion medium is/are added to the dispersion medium in such a way that the amount of highly mobile stabilizers is, after emulsification, sufficient to stabilize newly created surfaces during recrystallization. Optionally, one or more lipid-soluble or -dispersible stabilizers is/are added additionally to  
15 the molten agent or mixture of agents,
  - d. the molten agent or mixture of agents and the dispersion medium is/are emulsified in a liquid phase by high-pressure homogenization, sonication, high-speed stirring, vortexing  
20 and/or hand shaking, preferably by high-pressure homogenization,
  - e. the homogenized dispersion is allowed to cool until solid particles are formed by recrystallization of the dispersed agents.  
25
2. A process according to claim 1, c h a r a c t e r i z e d in that the homogenized dispersion is passed through a filter prior to cooling below the recrystallization temperature to remove particulate contaminants in such a way that the filter pore size is chosen large  
30 enough not to retain the particles of emulsified, molten agents.
3. A process according to claim 1 or 2, c h a r a c t e r i z e d in that the agent or mixture of agents is/are a lipid/lipids preferably having melting points between approximately 30°C and 120°C, preferably

**SUBSTITUTE SHEET**

constituted of mono-, di- and triglycerides of long chain fatty acids; hydrogenated vegetable oils; fatty acids and their esters; fatty alcohols and their esters and ethers; natural or synthetic waxes such as beeswax and carnauba wax; wax alcohols and their esters, sterols such as cholesterol and its esters, hard paraffins or mixtures of the above mentioned lipids.

- 5
4. A process according to claim 1 or 2, characterized in that the agent or mixture of agents is/are a bioactive agent/bioactive agents or drug/drugs preferably showing a low bioavailability and/or
- 10 being badly absorbed from the intestinum, having melting points preferably below 100°C or the melting points of which can be decreased to below 100°C by addition of adjuvants, preferably anesthetics and narcotics such as butanilcaine, fomocaine, isobutambene, lidocaine, risocaine, pseudococaine, prilocaine, tetracaine, trime-
- 15 caine, tropacocaine, and etomidate; anticholinergics such as metixen and profenamine; antidepressives, psychostimulants and neuroleptics such as alimenazine, binedaline, perazine, chlorpromazine, fempentadiol, fenanisol, mebenazine, methylphenidate, thioridazine, toloxaton and trimipramide; antiepileptics such as dimethadion and nicethamide;
- 20 antimycotics such as butoconazole, chlorphenesin, etisazole, exalamid, precilocine and miconazole; antiphlogistics such as butibufen and ibuprofen; bronchodilators such as bamifylline; cardiovascular drugs such as alprenolol, butobendine, clordiazole, hexobendine, nicofibrate, penbutolol, pirmenol, prenylamine, procaine amide, propatrylnitrate, suloctidil, toliprolol, xidbendol and viquidile; cytostatics
- 25 such as asperline, chlorambucile, mitotane, estramustine, taxol, penclomedine and trofosfamide; hyperemic drugs such as capsaicine and methylnicotinate, lipid reducers such as nicoclonate, oxprenolol pirifibrate, simfibrate and thiadenol; spasmolytics such as aminopromazine, caronerine, difemerine, fencarbamide, tiropramide and
- 30 moxaverine; testosterone derivatives such as testosterone enantate and testosterone-(4-methylpentanoate); tranquilizers such as azaperone and buramate; virustatics such as arildon; vitamin A derivatives

such as retinol, retinol acetate and retinol palmitate; vitamin E derivatives such as tocopherol acetate, tocopherol succinate and tocopherol nicotinate; menadione; cholecalciferol; insecticides, pesticides and herbicides such as acephate, cyfluthrin, azinphosphomethyl, cypermethrine, substituted phenyl thiophosphates, fenclaphos, permethrine, piperonal, tetramethrine and/or trifluraline.

- 5
5. A process according to anyone of the preceding claims, characterized in that the surface characteristics of the particles are modified after homogenization in order to control the biodistribution of the particles, preferably by adsorption of polymers or by dialysis of water-soluble surfactants.
- 10
6. A process according to anyone of the preceding claims, characterized in that during cooling the dispersion is agitated, such as by means of a magnetic stirrer.
- 15
7. A process according to anyone of the preceding claims, characterized in that the dispersion medium is a pharmacologically acceptable liquid not dissolving the agent or mixture of agents, especially water, ethanol, propylene glycol, dimethyl sulfoxide (DMSO) or methyl-isobutyl-ketone, or a mixture thereof, preferably
- 20
- water.
8. A process according to anyone of the preceding claims, characterized in that the stabilizer or stabilizers are amphiphatic compounds, especially ionic and non-ionic surfactants, preferably naturally occurring as well as synthetic phospholipids, their hydrogenated derivatives and mixtures thereof, sphingolipids and glycosphingolipids; physiological bile salts such as sodium cholate, sodium dehydrocholate, sodium deoxycholate, sodium glycocholate and sodium taurocholate; saturated and unsaturated fatty acids or fatty alcohols; ethoxylated fatty acids or fatty alcohols and their
- 25
- esters and ethers; alkylaryl-polyether alcohols such as tyloxapol; esters and ethers of sugars or sugar alcohols with fatty acids or fatty alcohols; acetylated or ethoxylated mono- and diglycerides; synthetic biodegradable polymers like block co-polymers of polyoxyethylene
- 30

and polyoxypropyleneoxide; ethoxylated sorbitanesters or sorbitanethers; amino acids, polypeptides and proteins such as gelatine and albumin; or a combination of two or more of the above mentioned stabilizers.

- 5      9.      A process according to anyone of the preceding claims,  
characterized in that the stabilizer or stabilizers is/are a  
combination of phospholipids and bile salts.
10.      A process according to claim 9, characterized in that the  
molar ratio of phospholipids to bile salts is 2:1 or above.
- 10      11.      A process according to anyone of claims 1-8, characterized  
in that the stabilizer or stabilizers is/are a combination of phospho-  
lipids and sodium glycocholate in a molar ratio between 2:1 and 4:1.
12.      A process according to anyone of claims 9-11,  
characterized in that the dispersion medium contains iso-  
15      tonic agents, preferably glycerol, and/or cryoprotectants, preferably  
sugars or sugar alcohols.
13.      A process according to anyone of the preceding claims,  
characterized in that the dispersion medium contains one  
or more of the following additives: water-soluble or dispersable  
20      stabilizers; isotonicity agents, preferably glycerol or xylitol; cryo-  
protectants, preferably sucrose, glucose, maltose or trehalose; elec-  
trolytes; buffers; antiflocculants, preferably sodium citrate, sodium  
pyrophosphate or sodium dodecylsulfate; and preservatives.
14.      A process according to anyone of the preceding claims,  
25      characterized in that the dispersion is sterilized, preferably  
by autoclaving or sterile filtration prior to cooling down the dispersion  
below the recrystallization temperature of the molten lipids.
15.      A process according to anyone of the preceding claims,  
characterized in that in a subsequent step the dispersion  
30      medium is reduced in volume, preferably by evaporation, or removed  
by filtration, ultrafiltration or freeze-drying, thus yielding liquid-free  
particles which can be reconstituted prior to use.

16. A suspension of colloidal solid lipid particles (SLPs) manufactured according to anyone of the claims 1-3 and 5-15, characterized in that the SLPs are lipids having melting points between approximately 30°C and 120°C, preferably constituted of mono-, di- and triglycerides of long chain fatty acids; hydrogenated vegetable oils; fatty acids and their esters; fatty alcohols and their esters and ethers; natural or synthetic waxes such as beeswax and carnauba wax; wax alcohols and their esters, sterols such as cholesterol and its esters, hard paraffins or mixtures of the above mentioned lipids.
17. A suspension of colloidal solid lipid particles (SLPs) according to claim 16, characterized in that the particles are stabilized by a combination of phospholipids and bile salts.
18. A suspension of colloidal solid lipid particles (SLPs) according to claim 17, characterized in that the molar ratio of phospholipids to bile salts is 2:1 or above.
19. A suspension of colloidal solid lipid particles (SLPs) according to claim 16, characterized in that the particles are stabilized by a combination of phospholipids and sodium glycocholate in a molar ratio between 2:1 and 4:1.
20. A suspension of colloidal solid lipid particles (SLPs) according to anyone of claims 17-19, characterized in that the dispersion medium contains isotonicity agents, preferably glycerol, and/or cryoprotectants, preferably sugars or sugar alcohols.
21. A suspension of colloidal solid lipid particles (SLPs) according to anyone of claims 16-20, characterized in that the SLPs are of a non- $\alpha$ -like crystalline modification at a temperature below the melting temperature.
22. A suspension of colloidal solid lipid particles (SLPs) according to anyone of claims 16-21, characterized in that the SLPs are of a non-spherical shape at a temperature below the melting temperature.

23. A suspension of colloidal solid lipid particles (SLPs) according to anyone of claims 16-22, characterized in that the particles are of micron or submicron size, predominantly in the size range from 20 to 500 nm.
- 5 24. A suspension of colloidal solid lipid particles (SLPs) according to anyone of claims 16-23, characterized in that into the SLPs are entrapped drugs or bioactive compounds which are preferably poorly water-soluble, show a low bioavailability, are badly absorbed from the intestine and/or will be rapidly degraded in  
10 biological environment by chemical or enzymatical processes.
25. A suspension of colloidal solid lipid particles (SLPs) according to claim 24, characterized in that the entrapped drugs are preferably antibiotics such as fosfomycin, fosmidomycin and rifapen-  
15 tin; antihypertensives such as minoxidil, dihydroergotamine and endralazine; antihypotensives such as dihydroergotamine; systemic antimycotics such as ketoconazole and griseofulvin; antiphlogistics such as indomethacin, diclofenac, ibuprofen, ketoprofen and piropro-  
20 fen; antiviral agents such as aciclovir, vidarabin and immunoglobu- lines; ACE inhibitors such as captopril and enalapril; betablockers such as propranolol, atenolol, metoprolol, pindolol, oxprenolol and labetalol; bronchodilators such as ipratropiumbromide and salbutamol;  
25 calcium antagonists such as diltiazem, flunarizin, verapamil, nifedipin, nimodipin and nitrendipin; cardiac glycosides such as digitoxin, di- goxin, methyl digoxin and acetyldigoxin; cephalosporins such as ceftizoxim, cefalexin, cefalotin and cefotaxim; cytostatics such as chlormethin, cyclophosphamid, chlorambucil, cytarabin, vincristin, mitomycin C, doxorubicin, bleomycin, cisplatin, taxol, penclomedine and estramustine; hypnotics such as flurazepam, nitrazepam and lorazepam; psychotropic drugs such as oxazepam, diazepam and  
30 bromazepam; steroid hormones such as cortisone, hydrocortisone, prednisone, prednisolone, dexamethasone, progesterone, pregnano- lone, testosterone and testosteroneundecanoat; vasodilators such as molsidomin, hydralazin and dihydralazin; cerebral vasodilators such

as dihydroergotoxin, ciclonicat and vincamin; lipophilic vitamins such as Vitamins A, D, E, K and their derivatives.

26. A suspension of particles of bioactive agents (PBAs) manufactured according to anyone of the claims 1, 2 and 4-15,

5 c h a r a c t e r i z e d in that the PBAs are drugs showing a low bioavailability and/or being badly absorbed from the intestinum having melting points preferably below 100°C or the melting points of which can be decreased to below 100°C by addition of adjuvants, especially anesthetics and narcotics such as butanilicaine, fomo-  
10 caine, isobutambene, lidocaine, risocaine, prilocaine, pseudococaine, tetracaine, trimecaine, tropacocaine and etomidate; anticholinergics such as metixen and profenamine; antidepressives, psychostimulants and neuroleptics such as alimenazine, binedaline, perazine, chlor-  
15 promazine, fempentadiol, fenanisol, fluanisol, mebenazine, methyl-phenidate, thioridazine, toloxaton and trimipramine; antiepileptics such as dimethadion and nicethamide; antimycotics such as buto-  
conazole, chlorphenesin, etisazole, exalamid, pecilocine and mico-  
nazole; antiphlogistics such as butibufen and ibuprofen; bronchodila-  
20 tors such as bamifylline; cardiovascular drugs such as alprenolol, butobendine, cloridazole, hexobendine, nicofibrate, penbutolol, pirmenol, prenylamine, procaine amide, propatylnitrate, suloctidil, toliprolol, xibendol and viquidile; cytostatics such as asperline, chlor-  
ambucil, chlornaphhazine, mitotane, estramustine, taxol, penclo-  
medine and trofosfamide; hyperemic drugs such as capsaicine and  
25 methylnicotinate; lipid reducers such as nicoclonate, oxprenolol, pirifibrate, simfibrate and thiadenol; spasmolytics such as amino-  
promazine, caronerine, difemerine, fencarbamide, tiropramide and moxaverine; testosterone derivatives such as testosterone enantate and testosterone-(4-methylpentanoate); tranquilizers such as azape-  
30 rone and buramate; virustatics such as arildon; vitamin A derivatives such as retinol, retinol acetate and retinol palmitate; vitamin E derivatives such as tocopherol acetate, tocopherol succinate and tocophe-  
rol nicotinate; menadione; cholecalciferol; insecticides, pesticides

**SUBSTITUTE SHEET**



and herbicides such as acephate, cyfluthrin, azinphosphomethyl, cypermethrine, substituted phenyl thiophosphates, fenclophos, permethrine, piperonal, tetramethrine and trifluraline.

- 5 27. A suspension of particles of bioactive agents (PBAs) according to claim 26 or manufactured according to anyone of the claims 4-15, characterized in that the PBAs are water-insoluble at the temperature of emulsion preparation and will not be solubilized by the excess of stabilizers or form micelles by themselves the particle size of PBAs remaining unchanged before and after cooling to room temperature.
- 10 28. A suspension of particles of bioactive agents (PBAs) according to claim 26 or manufactured according to anyone of claims 4-15, characterized in that the PBAs are partly water-soluble at the temperature of emulsion preparation and/or are able to form mixed micelles by the excess of stabilizers and/or form micelles by themselves leading to an increase of particle size after cooling to room temperature due to crystal growth and/or precipitation of dissolved bioactive agent and/or due to mass transport from smaller to larger particles.
- 15 29. Liquid-free particles manufactured by removing the dispersion medium from a suspension according to anyone of claims 16-25, preferably by filtration, ultrafiltration or freeze-drying.
- 20 30. Liquid-free particles manufactured by removing the dispersion medium from a suspension according to anyone of claims 26-28, preferably by filtration, ultrafiltration or freeze-drying.
- 25 31. Use of a suspension according to anyone of claims 16-28 or liquid-free particles according to claim 29 or 30, but not containing insecticides, pesticides or herbicides, for therapeutic purposes in a living human or animal body.
- 30 32. A suspension according to anyone of the claims 16-28 or liquid-free particles according to claim 29 or 30 for use as a medicament.



Fig. 1

2/12

## DIFFERENTIAL SCANNING CALORIMETRY

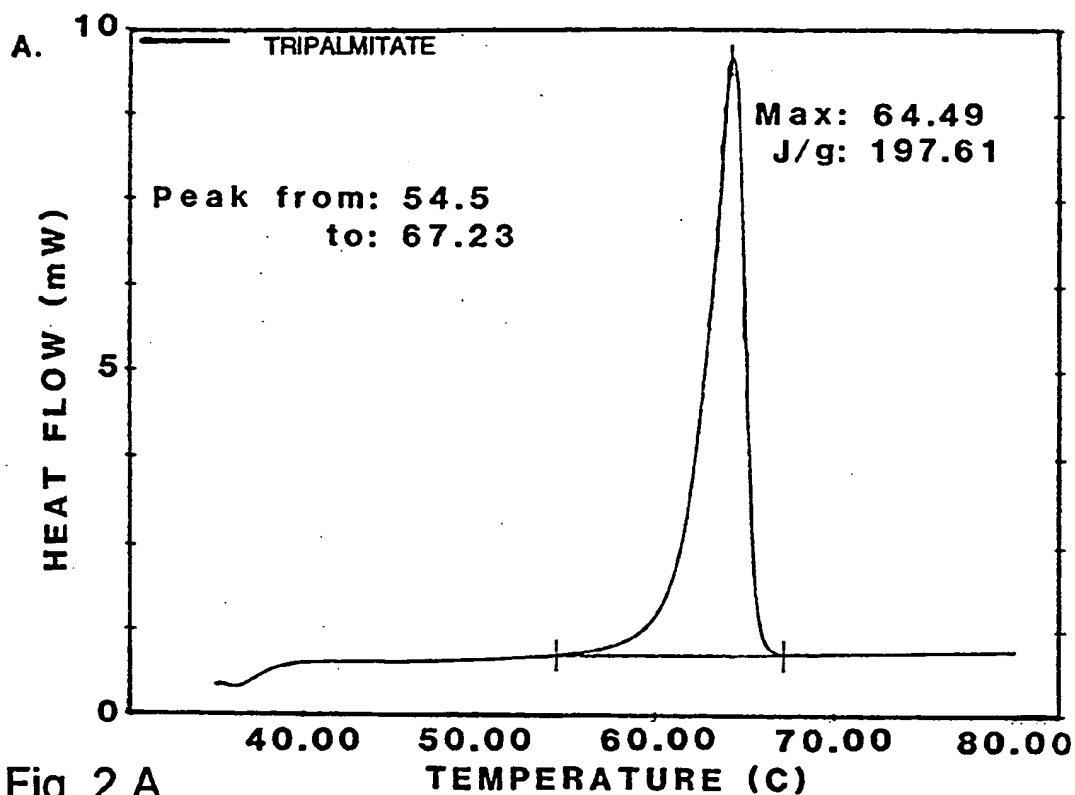


Fig. 2 A

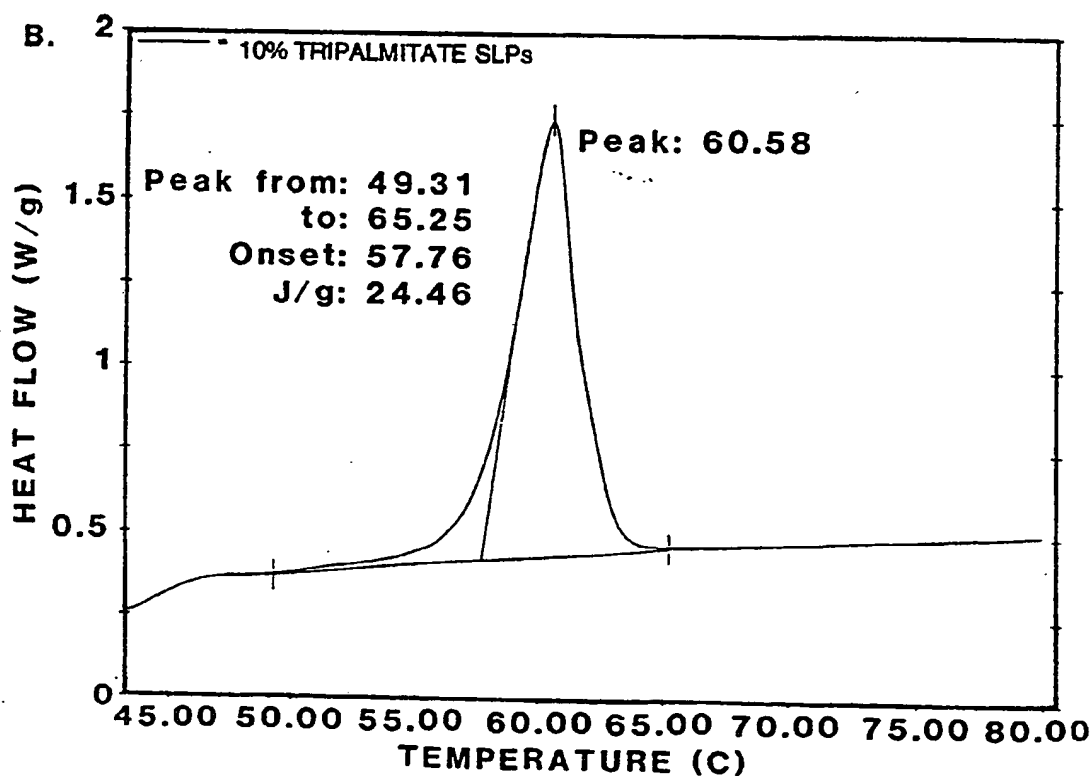


Fig. 2 B

SUBSTITUTE SHEET

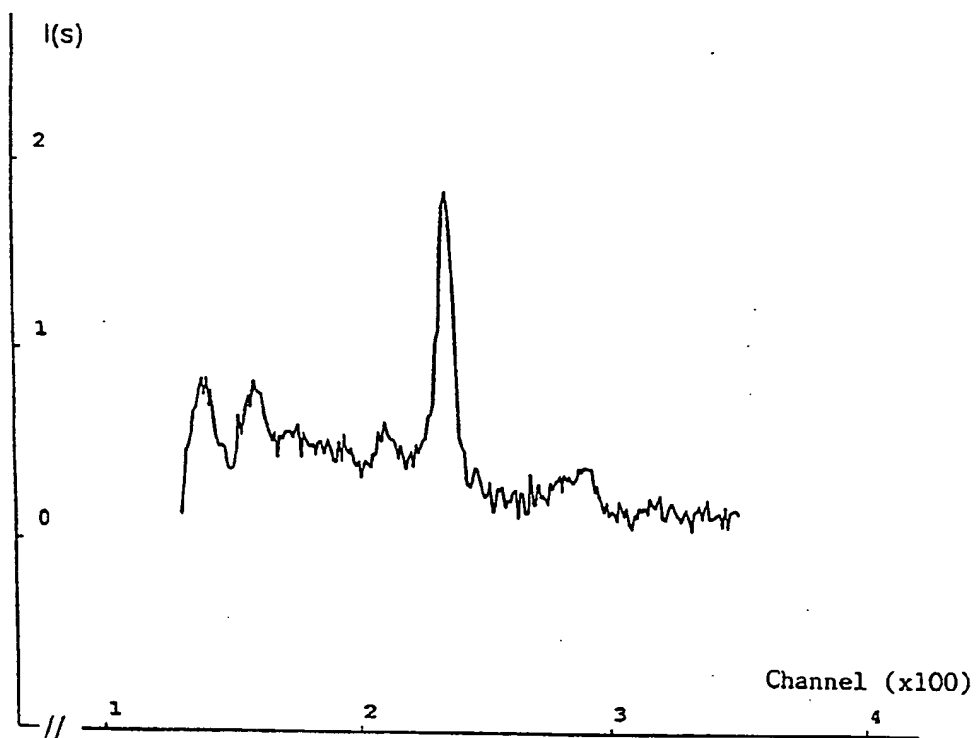


Fig. 3

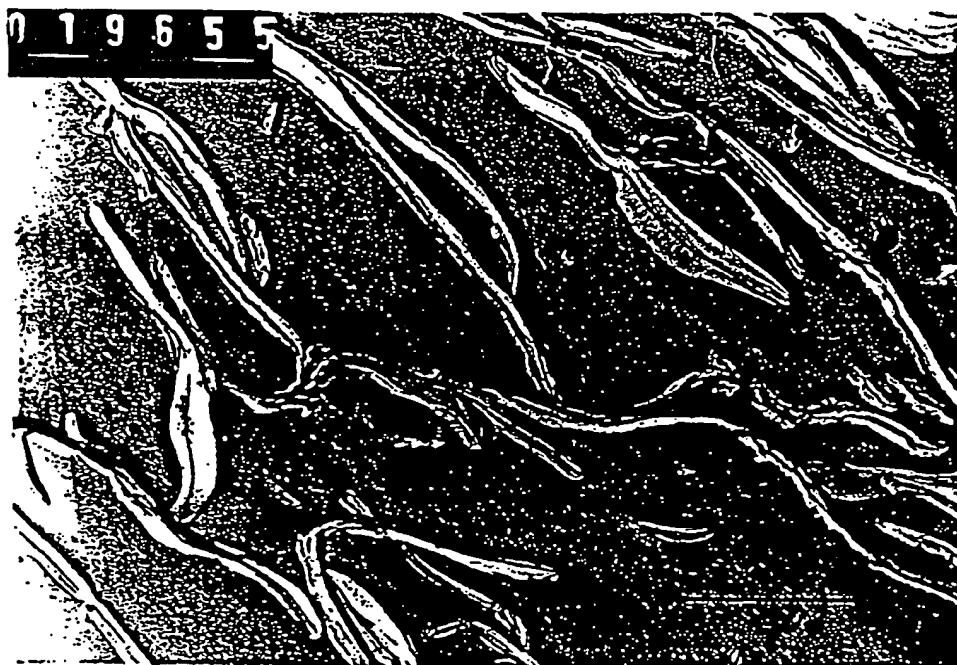


Fig. 4

# Malvern Multi-Angle PCS data analysis

10% Tripalmitate SLP dispersion

Angles 50/ 70/ 90/ 110/ 130/ degrees

File m19103ma.mad

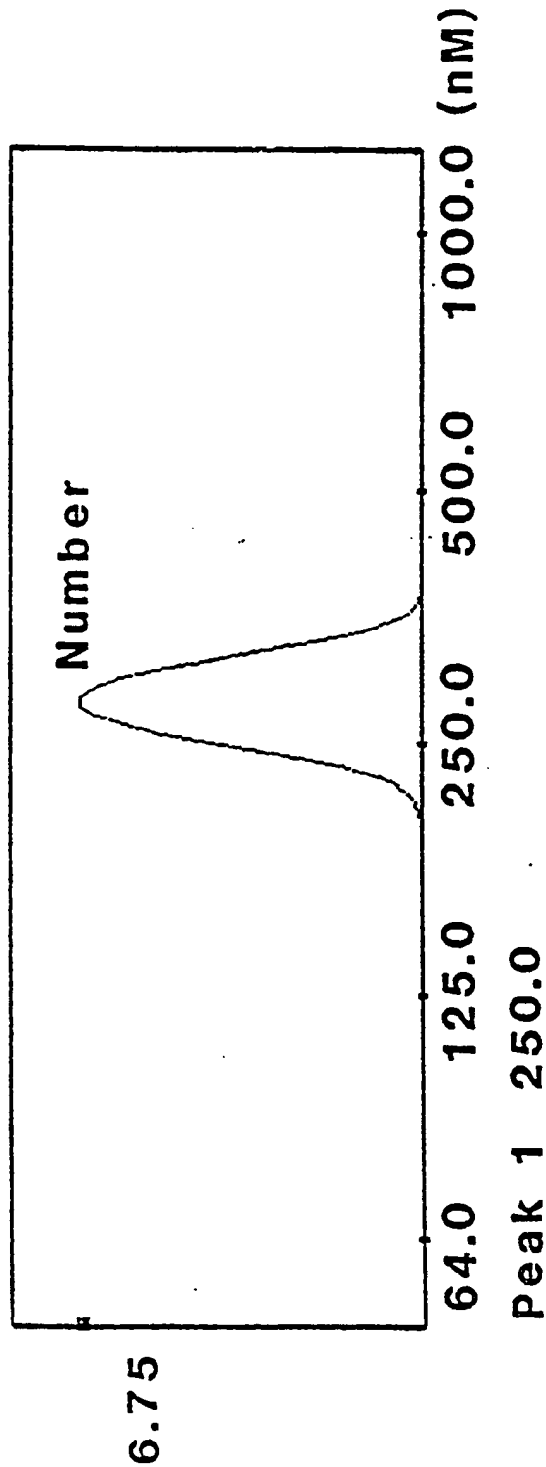


Fig. 5

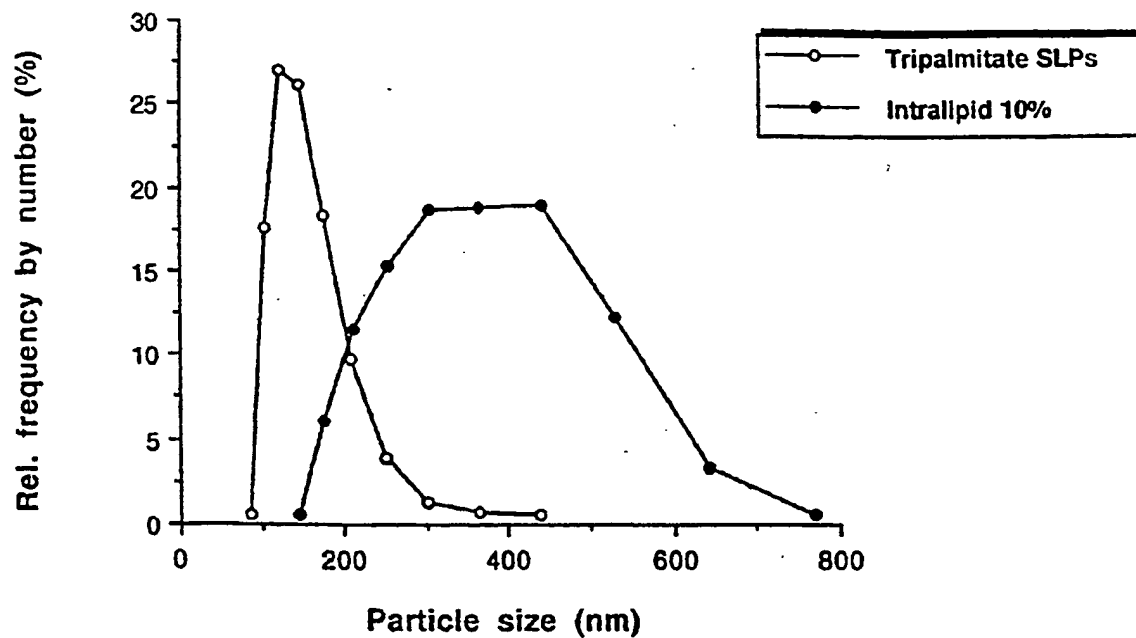


Fig. 6

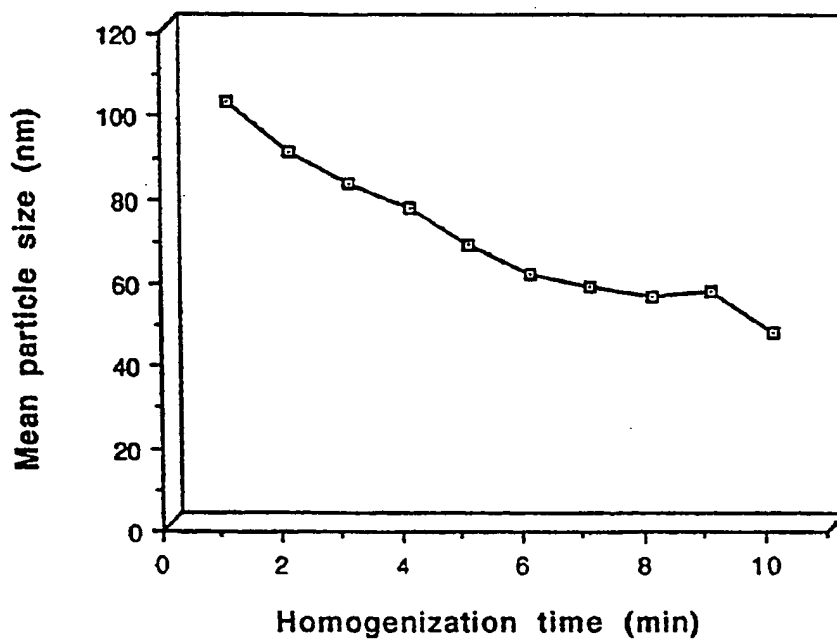


Fig. 7

SUBSTITUTE SHEET

6/12

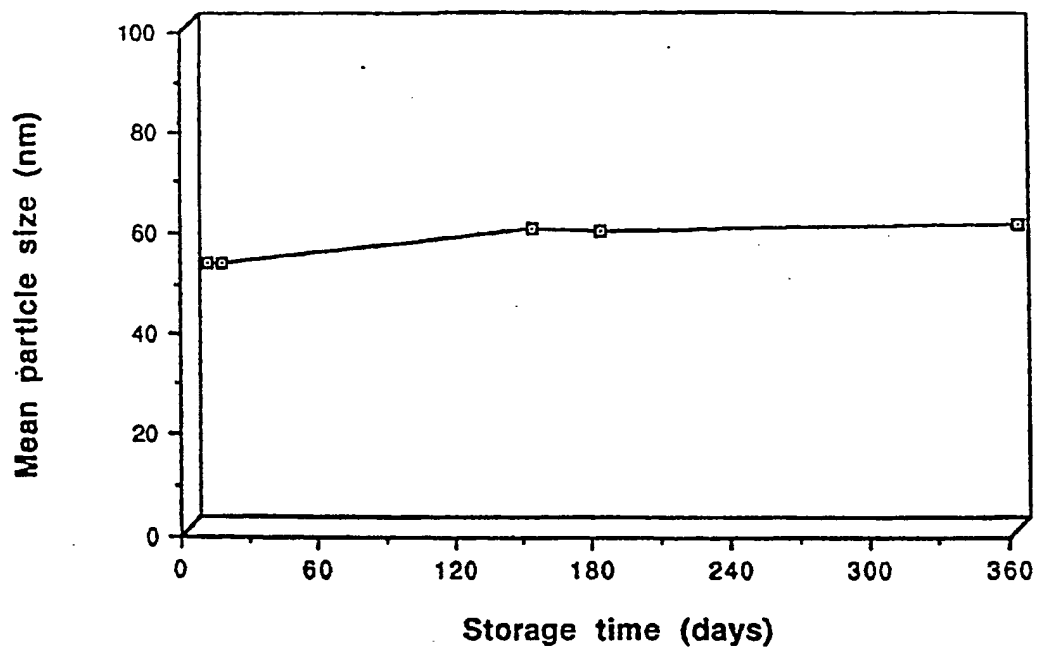


Fig. 8

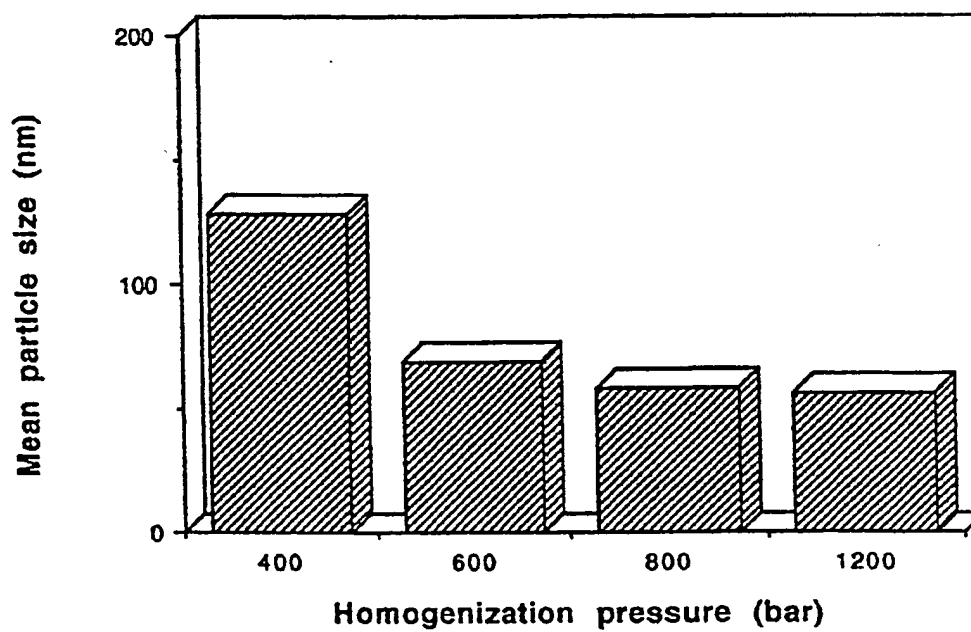


Fig. 9

SUBSTITUTE SHEET

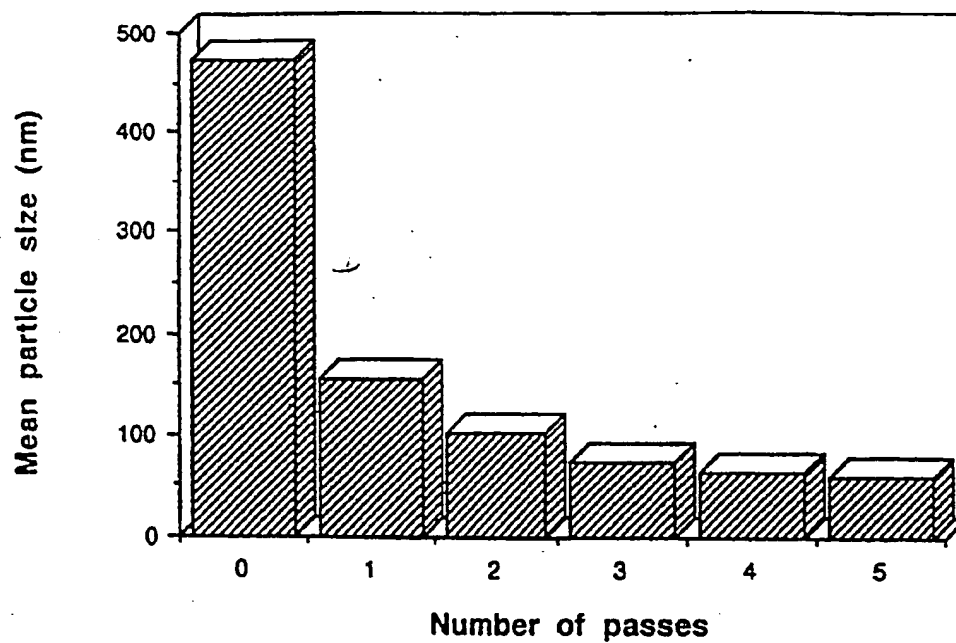


Fig. 10

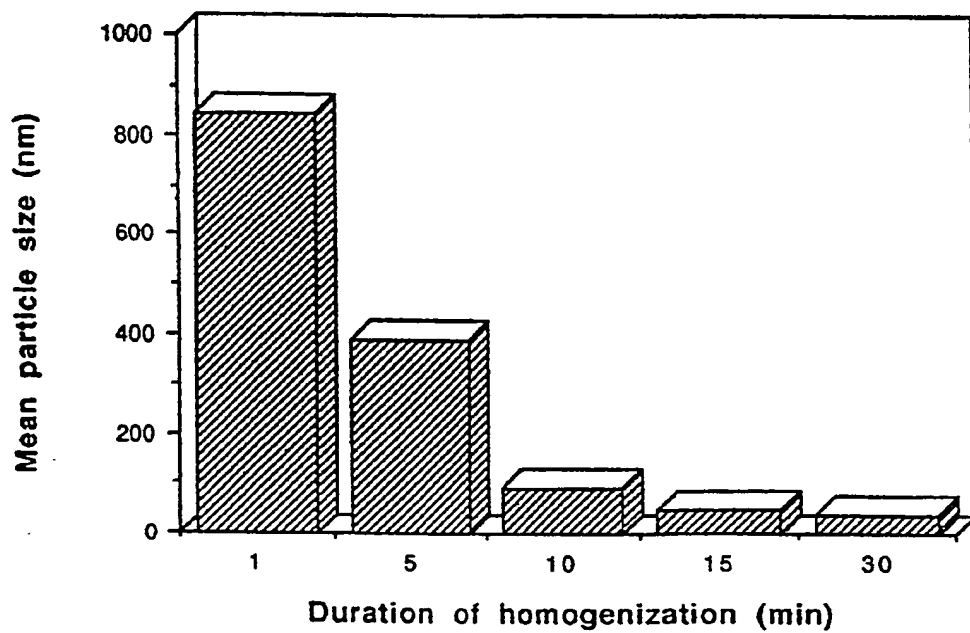


Fig. 11

SUBSTITUTE SHEET



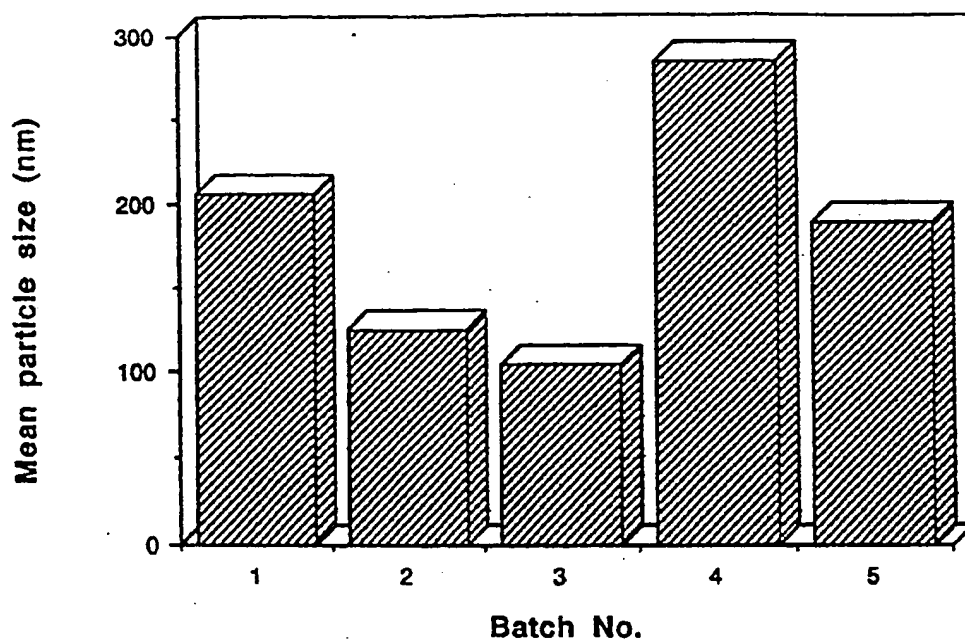


Fig. 12

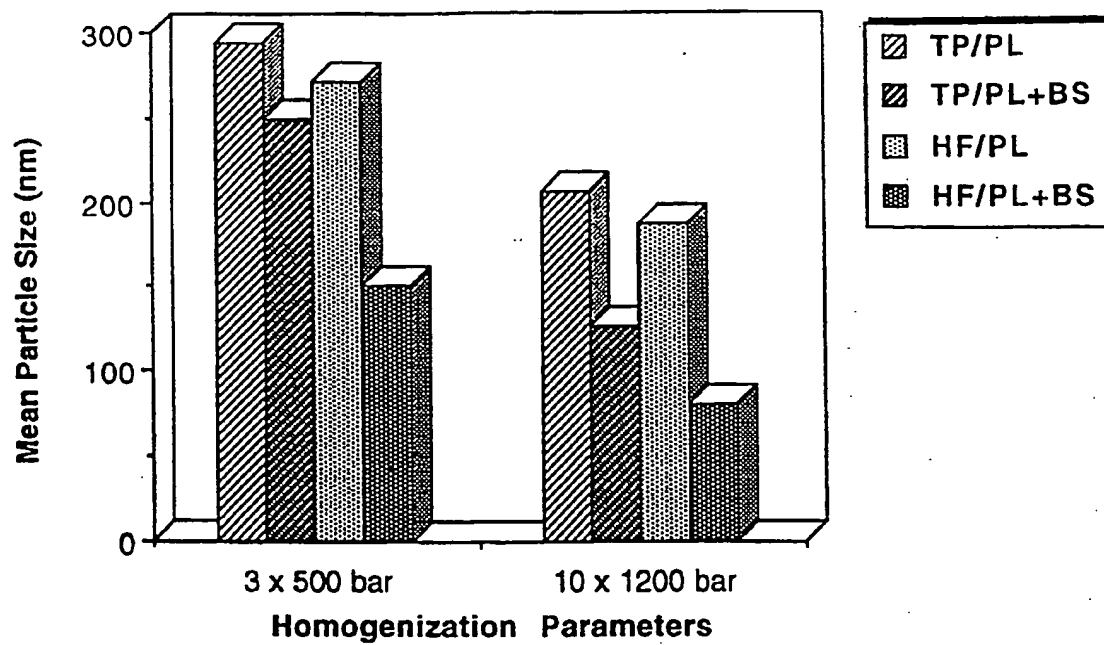


Fig. 13

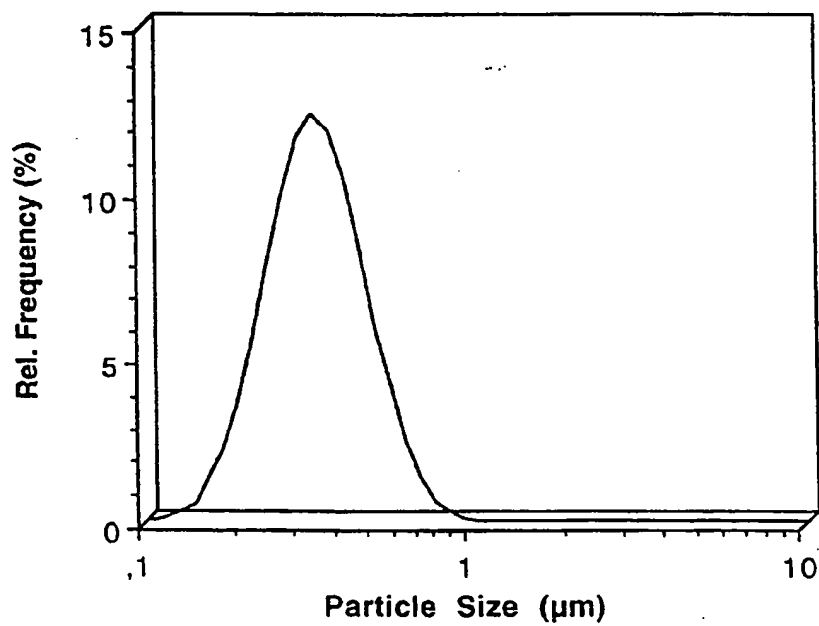


Fig. 14

SUBSTITUTE SHEET

10/12

## SYNCHROTRON RADIATION WIDE ANGLE X-RAY DIFFRACTION

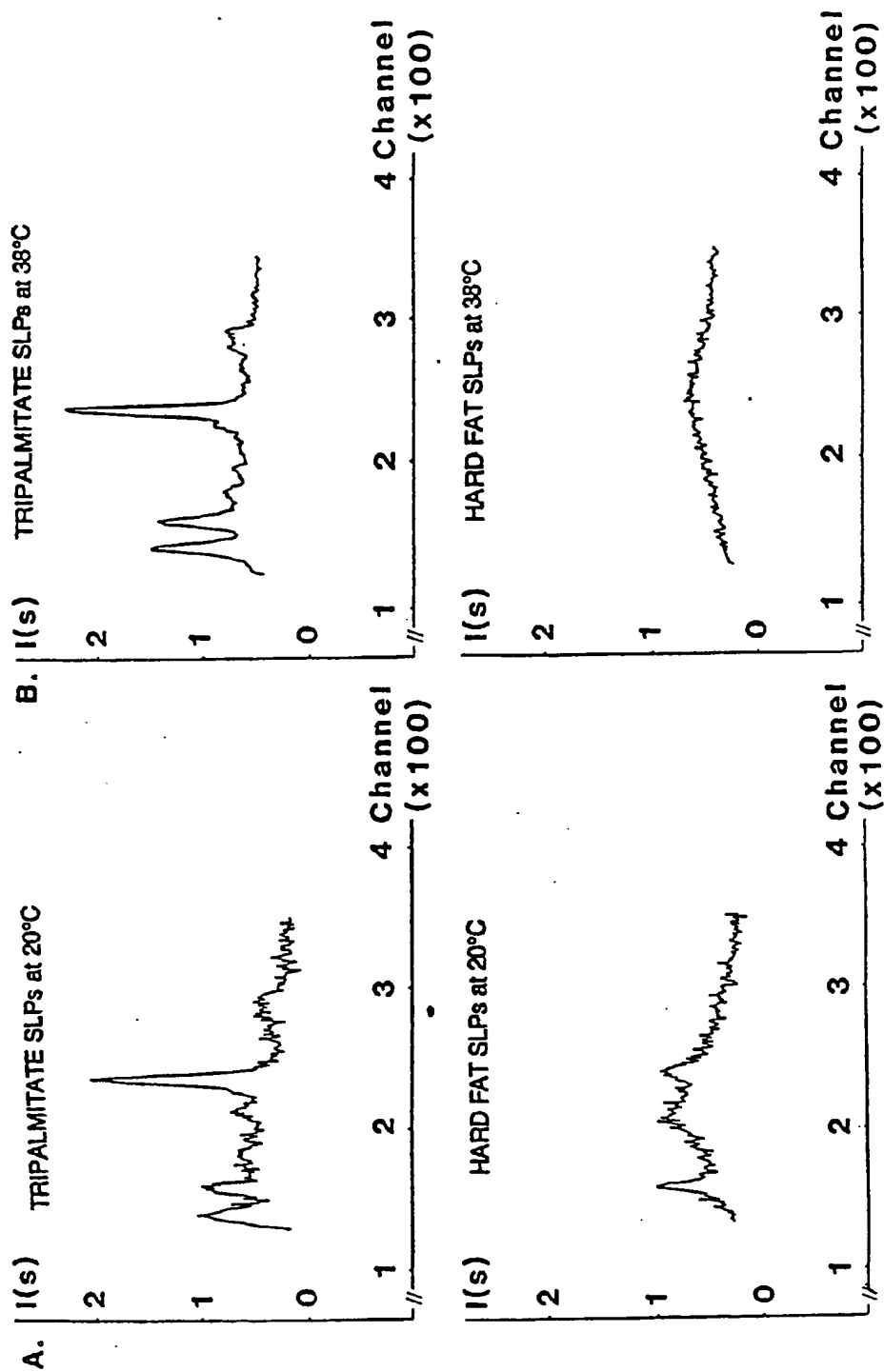


Fig. 15

SUBSTITUTE SHEET

11/12

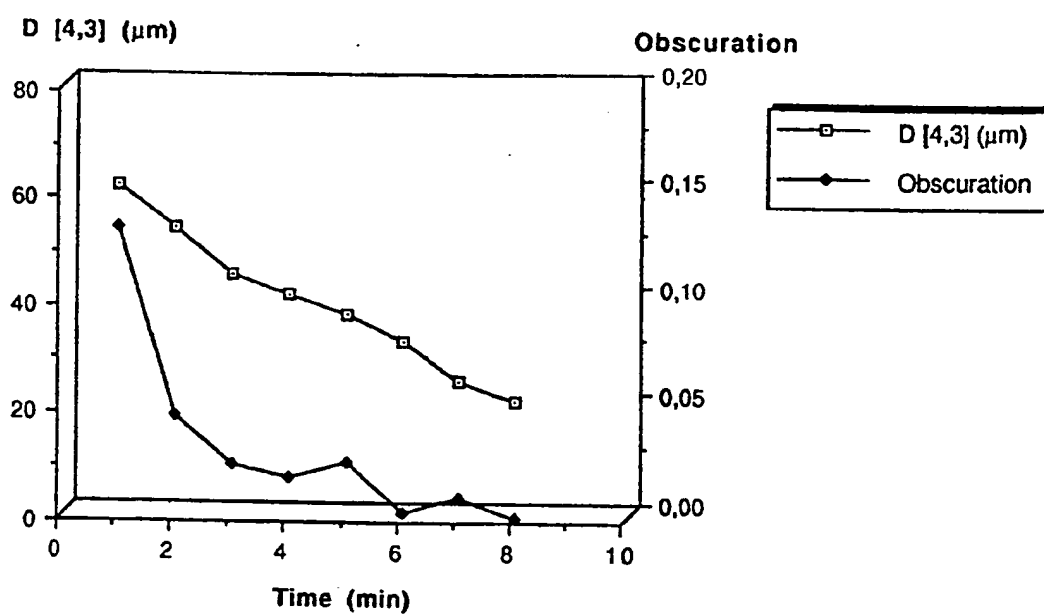


Fig. 16

SUBSTITUTE SHEET

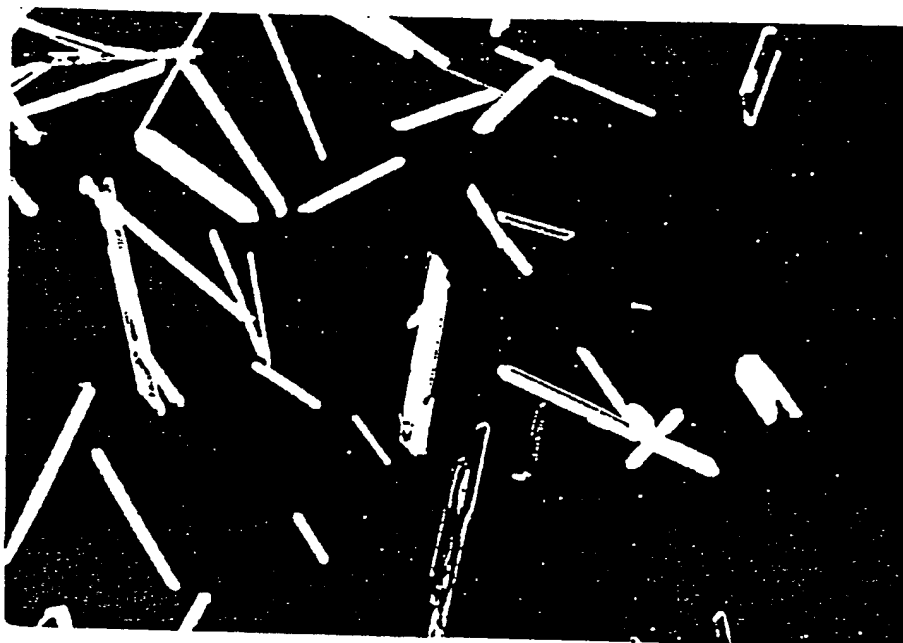


Fig. 17

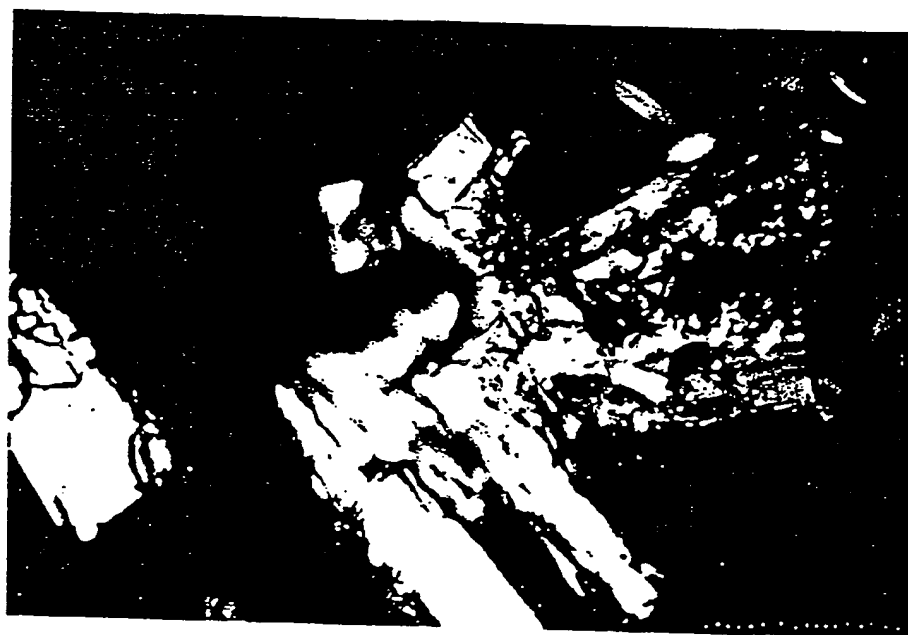


Fig. 18

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00185

## A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 9/10, A61K 9/16

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, B01F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EMBASE, CA SEARCH, MEDLINE, WPI, WPIL, CLAIMS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A1, 9107171 (NOVA PHARMACEUTICAL CORPORATION), 30 May 1991 (30.05.91), page 7 - page 22, claims  --	1-32
X	EP, A2, 0167825 (DR. RENTSCHLER ARZNEIMITTEL GMBH & CO), 15 January 1986 (15.01.86), page 14, line 13 - page 16, line 12, claims  --	1-32

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 June 1994

Date of mailing of the international search report

29 -06- 1994

Name and mailing address of the ISA/  
Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Anneli Jönsson  
Telephone No. +46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00185

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>Dialog Information Services, File 73, Embase, .  Dialog accession no. 8396396, Embase accession  no. 92072268, Bodmeier R. et al, "Process and  formulation variables in the preparation of  wax microparticles by a melt dispersion technique.  I. Oil-in-water technique for water-insoluble  drugs". J. Microencapsulation (United Kingdom),  1992, 9/1 (89-98)</p> <p style="text-align: center;">--</p>	1-32
A	<p>US, A, 5118511 (DIETER HORN ET AL), 2 June 1992  (02.06.92)</p> <p style="text-align: center;">--  -----</p>	1-32

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

28/05/94

International application No.  
PCT/SE 94/00185

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO-A1-	9107171	30/05/91	AU-A-	6950091	13/06/91
			CA-A-	2068216	14/05/91
			EP-A-	0502119	09/09/92
EP-A2-	0167825	15/01/86	SE-T3-	0167825	
			DE-A-	3421468	19/12/85
			JP-A-	61056122	20/03/86
			US-A-	4880634	14/11/89
US-A-	5118511	02/06/92	DE-A-	3702029	04/08/88
			DE-A-	3869629	07/05/92
			EP-A,B-	0276735	03/08/88
			SE-T3-	0276735	